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CORRECTIONS

Volume 38, 1960

Page 1320, line 2. For "Calc. for $C_{26}H_{47}O_2N$", substitute "Calc. for $C_{26}H_{47}O_3N$".

Page 1320, line 4. For "Calc. for $C_{26}H_{45}ON$", substitute "Calc. for $C_{26}H_{45}O_2N$".

Page 1320, last line of section on N-*Methoxy-4-aza-5β-cholestan-3-one*. For "Calc. for $C_{27}H_{45}O_2N$: C, 77.46; H, 11.55", substitute "Calc. for $C_{27}H_{47}O_2N$: C, 77.64; H, 11.35".

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THE SYNTHESIS OF ACETAMIDO-DEOXY KETOSES BY ACETOBACTER SUBOXYDANS

PART I[1]

J. K. N. JONES, M. B. PERRY, AND J. C. TURNER

ABSTRACT

The microbiological oxidation of 2-acetamido-2-deoxy-D-glucitol by *Acetobacter suboxydans* yielded a crystalline ketose which is shown to be 5-acetamido-5-deoxy-L-xylohexulose.

INTRODUCTION

The oxidation of unsubstituted polyhydric alcohols by proliferating cells of *Acetobacter suboxydans* has been studied intensively (1, 2, 3, 4, 5, 6), and has given rise to the Bertrand–Hudson rule for oxidations occurring in the pH range 5 to 6.5 (7, 8). Thus, a polyhydric alcohol possessing the D-erythro configuration (I) is oxidized mainly at the secondary alcohol group adjacent to the terminal primary alcohol to give a ketose (II). Bollenback and Underkofler (9), Richtmyer, Stewart, and Hudson (10), Hough, Jones, and Mitchell (11), and Jones and Mitchell (12) investigated the microbiological oxidation of terminal-substituted polyhydric alcohols. The latter authors reported the oxidation of several alcohols, giving chromatographic evidence for the presence of oxidation products, and characterizing completely the ketoses produced from 1-deoxy-1-*S*-ethyl-D-glucitol (11) and 1-deoxy-1-*S*-ethyl-D-arabitol (12).

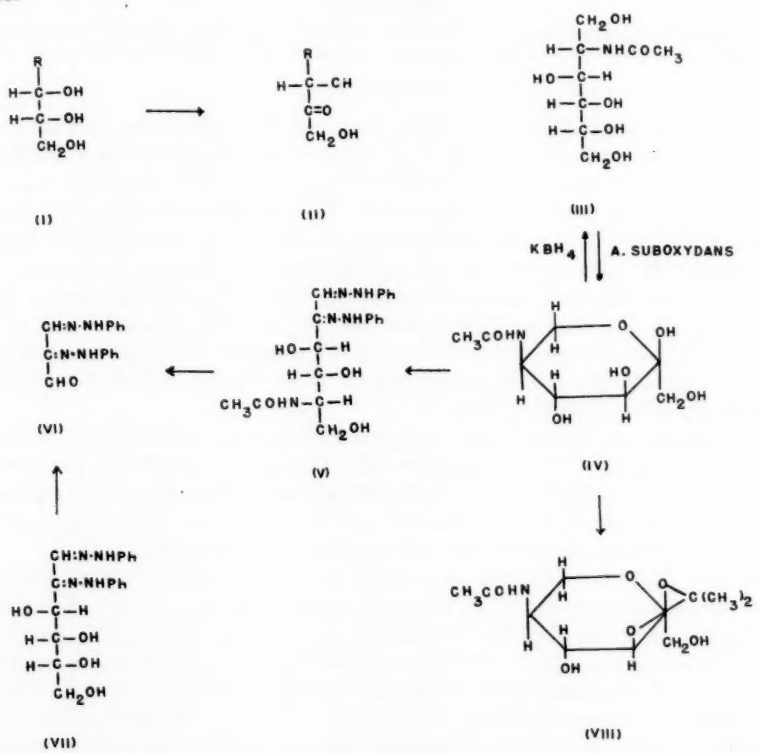
We now wish to report the isolation and proof of structure of the ketose 5-acetamido-5-deoxy-L-xylohexulose (IV), produced by microbiological oxidation at pH 5 to 6.5 of 2-acetamido-2-deoxy-D-glucitol (III). Oxidation was slow when the culture medium contained only 2-acetamido-2-deoxy-D-glucitol, and was more rapid when glycerol (0.3%) was included. However, oxidation was not complete after 21 days.

The product (IV) was obtained as a white crystalline solid. Analysis indicated an acetamido-deoxy hexulose and the compound gave absorptions in the infrared corresponding to OH, NH, and the amide linkage. On periodate oxidation of (IV) in unbuffered solution it was found to consume 2.85 moles of periodate with the liberation of 0.85 mole formic acid and 0.8 mole formaldehyde per mole of sugar, which indicated that the ketose (IV) possessed a pyranose ring form. The ketose (IV) gave a crystalline phenylosazone (5-acetamido-5-deoxy-L-xylohexose phenylosazone) (V, acyclic form) which possessed an acetamido-deoxy group and gave absorptions in the infrared corresponding to OH, NH, the aromatic ring, and the amide linkage. The phenylosazone (V), when oxidized with periodate by the method of Hough, Powell, and Woods (13), consumed 1 mole of periodate immediately and released no formic acid or formaldehyde. Periodate oxidation gave an immediate precipitate of the 1,2-bisphenylhydrazone of mesoxalaldehyde (VI) (14),

[1]*Manuscript received January 12, 1961.*
Contribution from the Department of Chemistry, Queen's University, Kingston, Ontario.

which was identified by mixed melting point and comparison of its infrared spectrum with that of an authentic sample obtained from the periodate oxidation of D-glucose phenylosazone (VII, acyclic form). These results indicated that the osazone (V) possessed hydroxyl groups at carbons 3 and 4 and that the acetamido-deoxy group was located at carbon 5. Considered with the preceding evidence, these results indicate that the site of biological oxidation in 2-acetamido-2-deoxy-D-glucitol (III) was at carbon 5, in accordance with the well-known enzyme specificity for oxidations at pH 5 to 6.5.

The ketose (IV) was shown to be a derivative of L-sorbose by its reduction with sodium borohydride to 2-acetamido-2-deoxy-D-glucitol, which had melting point, optical rotation, and infrared spectrum identical with those of an authentic specimen. The ketose (IV) gave, with acidified acetone, a non-reducing *O*-isopropylidene derivative (VIII) which was not attacked by periodate under unbuffered conditions, indicating that the mono-*O*-isopropylidene group spanned carbons 2 and 3 as in the case of 2,3-mono-*O*-isopropylidene-L-sorbose.



The microbiological oxidations of 1-acetamido-1-deoxy-*N*-methyl-D-glucamine and 1,2-dideoxy-2-acetamide-D-glucitol have been carried out and structural studies are in progress on the ketoses isolated.

EXPERIMENTAL

Solutions were concentrated under reduced pressure (ca. 15 mm) below 40° C. Melting points are uncorrected and optical rotations were determined in water at 23±3° C unless

otherwise stated. Paper chromatography was carried out on Whatman No. 1 paper using the descending method (15). The following solvent systems were used (v/v):

(*a*) ethyl acetate – acetic acid – formic acid – water (18:3:1:4);
(*b*) butan-1-ol – pyridine – water (5:3:2);
(*c*) butan-1-ol – ethanol – water (9:3:3).

Reducing compounds were detected with the *p*-anisidine hydrochloride spray reagent (16) and ketoses with the orcinol/trichloroacetic acid spray reagent (17). Non-reducing compounds were detected with the alkaline silver nitrate spray reagent (18). Rates of movement of the sugars on paper chromatograms are given relative to that of rhamnose (R_{rh}). Infrared spectra were measured as a 6% solution in chloroform or as a 0.8% dispersion in a potassium bromide disk, using a Perkin–Elmer Model 21 spectrophotometer.

Acetobacter suboxydans (A.T.C.C. No. 621) was obtained from the American type culture collection and maintained at 5° C on slopes of agar (3% w:v) containing sorbitol (5% w:v), yeast extract powder (0.5% w:v), potassium dihydrogen phosphate (0.05% w:v), and D-glucose (0.05% w:v). At approximately 2-month intervals a broth containing sorbitol (7% w:v), yeast extract powder (0.5% w:v), and potassium dihydrogen phosphate (0.05% w:v) in tap water was inoculated from the slopes. After 72 hours, when growth of the organism was apparent, fresh slopes were inoculated from the broth and left 2–3 days at room temperature when cream-colored colonies became visible. The slopes were then stored at 5° C.

5-Acetamido-5-deoxy-L-xylohexulose

A broth consisting of 2-acetamido-2-deoxy-D-glucitol (8.5 g), prepared by sodium borohydride reduction of 2-acetamido-2-deoxy-D-glucose, glycerol (0.5 g), yeast extract powder (0.8 g), and potassium dihydrogen phosphate (0.16 g) in tap water (160 ml) was distributed among eight 250-ml conical flasks giving a surface:volume ratio of ca. 2.5:1. The broths were then autoclaved at 15 p.s.i. for 30 minutes, allowed to cool, and each inoculated with one drop of a 48-hour culture of *Acetobacter suboxydans* grown in glycerol (6%). The broths were stored at room temperature in the dark, without agitation, for 21 days. At intervals small samples (2 ml) were withdrawn using sterile pipettes and the broth examined on paper chromatograms. The gradual disappearance of glycerol and the appearance of dihydroxyacetone (1,3-dihydroxypropanone) and a spot which had R_{rh} 0.59 (solvent (*c*)), 0.73 (solvent (*a*)), 0.65 (solvent (*b*)), and gave a yellow color with orcinol/trichloroacetic acid spray were noted. A quantitative measure of the rate of oxidation was obtained by estimation of the copper-reducing power of the broth using the Somogyi method (19). The results are recorded in Table I. After 21 days the broths

TABLE I

Time (days)	% yield of ketose (corrected for the presence of dihydroxyacetone)
3	4.5
11	11.25
17	14.55

were combined and poured into 2 volumes of ethanol. The cell debris was removed by filtration and the filtrate was evaporated to about 20 ml and then deionized by

passage through columns of Amberlite IR 120 (H) and Duolite A4 (OH) resins. The eluate was concentrated to a syrup which crystallized. The crystalline material was washed with ethanol and dried, m.p. 154–155° C, mixed m.p. with 2-acetamido-2-deoxy-D-glucitol, 154–155° C. The mother liquors from the crystallization were concentrated to a syrup (2.4 g) which was deposited on cellulose powder and packed as a level band on top of a cellulose column (54 cm×4.5 cm). The column was irrigated with butan-1-ol half saturated with water, and 5-ml fractions of the eluate were collected using an automatic fraction collector (20). The fractions were examined on paper chromatograms run in solvent (*c*) and sprayed with alkaline silver nitrate spray reagent. A good separation of the sugars was obtained and the appropriate fractions were evaporated to dryness yielding crystalline 5-acetamido-5-deoxy-L-xylohexulose. One recrystallization from ethanol/ether gave crystals m.p. 174–176° C, $[\alpha]_D$ −62. Anal. Calc. for $C_8H_{15}O_6N$: C, 43.4; H, 6.8; N, 6.3. Found: C, 43.3; H, 6.9; N, 6.35. Absorptions in the infrared due to OH and NH (broad fused peak at 3300 cm^{-1}) and the amide linkage (1625 cm^{-1} and 1570 cm^{-1}) were observed. For infrared spectrum see Appendix (A).

Periodate Oxidation of 5-Acetamido-5-deoxy-L-xylohexulose

The ketose (50.62 mg) was dissolved in about 40 ml of water, 0.3 *M* sodium metaperiodate (5.0 ml) was added, and the volume was made up to 50 ml with water. A blank was prepared with 0.3 *M* sodium metaperiodate (5.0 ml), which was made up to 50 ml with water. The solutions were stored in the dark at room temperature and 5-ml aliquots were removed at intervals from each of the two solutions for the determination of periodate uptake (21) and formic acid production (22). Formaldehyde production was measured by the chromotropic acid method (23). The results are recorded in Tables II and III.

TABLE II

Oxidation of 5-acetamido-5-deoxy-L-xylohexulose

Time (hr)	Periodate uptake (moles/mole)	Formic acid production (moles/mole)
0.12	1.15	0.31
1.83	1.79	0.59
8.5	2.68	0.82
24	2.85	0.85

TABLE III

Time (hr)	Formaldehyde production (moles/mole)
0.2	0.36
1	0.53
25	0.62
45	0.80

5-Acetamido-5-deoxy-L-xylohexose Phenylosazone

5-Acetamido-5-deoxy-L-xylohexulose (45 mg) was dissolved in water (2.4 ml), and acetic acid (0.24 ml) and freshly distilled phenylhydrazine (0.24 ml) were added. The mixture was heated in a boiling-water bath for 15 minutes, after which time the phenylosazone had crystallized. The solution was cooled and the phenylosazone was collected by filtration, washed with ice-cold dilute acetic acid, ice water, and cold (−60° C)

methanol, and then dried. One recrystallization from ethanol gave a yellow powder (small needles) m.p. 202–203° C (decomposes). (An optical rotation could not be measured due to the intense color of the osazone.) Anal. Calc. for $C_{20}H_{28}O_4N_6$: C, 60.15; H, 6.3; N, 17.5. Found: C, 59.7; H, 6.35; N, 17.3.

Absorptions in the infrared due to OH and NH (broad fused peak at 3300 cm^{-1}), the aromatic ring (3030 cm^{-1}, 1605 cm^{-1}, 1500 cm^{-1}, 745 cm^{-1}, 690 cm^{-1}), and the amide linkage (1647 cm^{-1}, 1583 cm^{-1}) were observed. The infrared spectrum is given in the Appendix (B).

*Periodate Oxidation of 5-Acetamido-5-deoxy-*L*-xylohexose Phenylosazone*

The method of Hough, Powell, and Woods (13) was used. The results are shown in Table IV. (Formaldehyde was estimated by the chromotropic acid method (23).)

TABLE IV

Time (hr)	Periodate uptake (moles/mole)	Formic acid (moles/mole)	Formaldehyde (moles/mole)
0.25	1.1	0.00	0.00
3.15	1.14	0.00	0.00
18.65	1.22	0.00	0.00
40.65	1.18	0.00	

On oxidation a bright yellow precipitate of the 1,2-bisphenylhydrazone of mesoxalaldehyde was obtained. The material was collected by filtration, washed with water, recrystallized from aqueous ethanol, and obtained as orange needles, m.p. 188–189.5° C.

1,2-Bisphenylhydrazone of Mesoxalaldehyde (14)

D-Glucose phenylosazone was prepared (300 mg) and dissolved in 50% aqueous ethanol (500 ml). Sodium metaperiodate (20 ml; 0.3 *M*) was added with vigorous stirring and after about 15 seconds a curdy mass of fine orange-yellow needles was precipitated. The mixture was allowed to stand for 15 minutes, then the crystals were collected by filtration, and washed with cold 50% aqueous ethanol and dried. One recrystallization from aqueous ethanol gave orange needles, m.p. 188.5–190.5° C.

The two specimens of the 1,2-bisphenylhydrazone of mesoxalaldehyde had mixed m.p. 188–189.5° C and had identical R_{rh} values on paper chromatograms run in solvents (*a*), (*b*), and (*c*) (R_{rh} 2.03, 1.58, and 2.9 respectively). The infrared spectra were identical and showed absorptions due to NH (3310 cm^{-1}), the aromatic ring (3050 cm^{-1}, 1605 cm^{-1}, 1500 cm^{-1}, 745 cm^{-1}, 687 cm^{-1}), and the unsaturated aldehyde group (1675 cm^{-1}). Further details of the infrared spectrum are given in the Appendix (C).

*Potassium Borohydride Reduction of 5-Acetamido-5-deoxy-*L*-xylohexulose to 2-Acetamido-2-deoxy-*D*-glucitol*

5-Acetamido-5-deoxy-L-xylohexulose (100 mg) was dissolved in water (10 ml) and the solution was cooled in ice. A solution of potassium borohydride (100 mg) in water (10 ml) was added dropwise, with stirring, over a period of 30 minutes and the solution was then stirred at 0° C for 3 hours, after which time it was non-reducing towards Fehling's solution. Amberlite IR-120 (H) resin was added to decompose excess potassium borohydride and the solution was then passed through a column of Amberlite IR-120 (H). The eluate and washings from the column were concentrated to a white amorphous residue which was repeatedly evaporated to dryness with methanol to remove boric acid as

methyl borate. The residue was dissolved in hot methanol and ether added to incipient turbidity. On cooling, white needles (57 mg) of 2-acetamido-2-deoxy-D-glucitol were deposited. A further crop of crystals (30 mg) was obtained on concentration of the mother liquors. The material had m.p. 154–156° C, mixed m.p. with an authentic specimen of 2-acetamido-2-deoxy-D-glucitol, 153–156° C, and $[\alpha]_D$ −10.8°. The crystalline material gave one spot when run on paper chromatograms in solvents (*a*), (*b*), and (*c*), detected with the alkaline silver nitrate reagent. The spot had R_{rh} values identical with those of the authentic specimen (solvent (*a*) 0.79; solvent (*b*) 0.62; solvent (*c*) 0.50). The infrared spectra of the derived and authentic specimens were identical and showed absorptions due to OH and NH (very broad peak at 3300 cm^{-1}) and the amide linkage (1645 cm^{-1}, 1587 cm^{-1}). Further details are given in the Appendix (D).

2,3-Mono-O-isopropylidene-5-acetamido-5-deoxy-L-xylohexulose

5-Acetamido-5-deoxy-L-xylohexulose (100 mg) was shaken with dry acetone (50 ml) containing concentrated sulphuric acid (2 drops) for 24 hours. At the end of this time the clear solution was neutralized with barium carbonate, filtered, and the filtrate was concentrated to a clear colorless syrup (120 mg) which was examined on paper chromatograms run in solvents (*a*), (*b*), and (*c*). The *p*-anisidine hydrochloride spray reagent showed only a very faint spot due to 5-acetamido-5-deoxy-L-xylohexulose. However, the orcinol/trichloroacetic acid spray reagent revealed also a strong spot due to the *O*-isopropylidene compound with the following R_{rh} values (Table V). An ethanol solution of

TABLE V

Solvent	R_{rh} (f = faint, s = strong)	
	p-Anisidine hydrochloride	Orcinol/trichloroacetic acid
(*a*)	0.73 (f)	0.73 (f)+1.86 (s)
(*b*)	0.65 (f)	0.65 (f)+1.73 (s)
(*c*)	0.59 (f)	0.59 (f)+1.95 (s)

the syrup was applied to Whatmann 3 mm paper and developed in solvent (*a*) overnight. The section containing the *O*-isopropylidene ketose was cut out and eluted with 50% aqueous ethanol. The eluate was passed through Amberlite IR-120 (H) and Duolite A-4 (OH) resins and then concentrated to a clear colorless syrup (108 mg) which was chromatographically pure. The syrup had $[\alpha]_D$ −51° (*c*, 2.16 in ethanol), and was non-reducing towards Fehling's solution. The infrared spectrum showed absorptions due to OH and NH (broad peak at 3360 cm^{-1}), the amide linkage (1665 cm^{-1}, 1560 cm^{-1}), and the C-methyl group (1460 cm^{-1}, 1380 cm^{-1}). A considerable reduction of the OH peak as compared to that of 5-acetamido-5-deoxy-L-xylohexulose was observed. Further details are given in the Appendix (E).

Periodate Oxidation of 2,3-Mono-O-isopropylidene-5-acetamido-5-deoxy-L-xylohexulose

The *O*-isopropylidene ketose (75 mg) was dissolved in 50% aqueous ethanol (ca. 40 ml). A 0.3 *M* quantity of sodium metaperiodate (2 ml) was added and the volume made up to 50 ml with 50% aqueous ethanol. A blank solution was prepared containing 0.3 *M* sodium metaperiodate (2 ml), which was made up to 50 ml with 50% aqueous ethanol. The solutions were stored in the dark at room temperature and 5-ml aliquots removed at intervals for estimation of periodate uptake (22). No periodate was taken up by the *O*-isopropylidene ketose.

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APPENDIX

Infrared Spectra

The following abbreviations are used: s = strong, m = medium, w = weak; wave numbers expressed in cm^{-1}.

*(A) 5-Acetamido-5-deoxy-*L*-xylohexulose (0.8% in KBr)*

3300 (s), 2950 (m), 2880 (m), 1625 (s), 1570 (s), 1510 (m), 1475 (m), 1440 (s), 1400 (m), 1380 (m), 1360 (m), 1330 (s), 1310 (m), 1280 (m), 1255 (m), 1225 (w), 1185 (m), 1160 (s), 1120 (s), 1100 (s), 1065 (s), 1030 (s), 955 (m), 905 (m), 825 (s), 785 (m), 685 (m), 670 (m).

*(B) 5-Acetamido-5-deoxy-*L*-xylohexose Phenylosazone (0.8% in KBr)*

3300 (s), 3030 (m), 2960 (m), 2910 (m), 1647 (s), 1605 (s), 1583 (s), 1540 (s), 1500 (s), 1450 (m), 1420 (m), 1385 (m), 1305 (m), 1257 (s), 1170 (m), 1150 (m), 1105 (m), 1055 (m), 1010 (s), 960 (w), 890 (w), 880 (w), 785 (w), 745 (m), 690 (m).

(C) 1,2-bis Phenylhydrazone of Mesoxalaldehyde (Authentic and Derived Specimens) (0.8% in KBr)

3310 (s), 3050 (w), 2840 (w), 1675 (s), 1605 (s), 1550 (s), 1535 (s), 1500 (s), 1355 (m), 1280 (s), 1245 (s), 1200 (m), 1170 (m), 1165 (m), 1155 (m), 745 (s), 705 (m), 687 (m).

*(D) 2-Acetamido-2-deoxy-*D*-glucitol (Authentic and Derived Specimens) (0.8% in KBr)*

3300 (s), 2940 (s), 1645 (s), 1587 (s), 1447 (s), 1385 (s), 1315 (s), 1277 (s), 1255 (m), 1215 (w), 1145 (w), 1130 (w), 1090 (s), 1065 (s), 1010 (s), 970 (m), 940 (w), 895 (w), 880 (m), 765 (m), 690 (s).

*(E) 2,3-Mono-O-isopropylidene-5-acetamido-5-deoxy-*L*-xylohexulose (6% in Chloroform)*
3360 (s), 3100 (w), 3030 (m), 2970 (w), 2910 (w), 1725 (w), 1665 (s), 1560 (s), 1460 (m), 1435 (m), 1390 (s), 1380 (s), 1315 (m), 1240 (s), 1190 (s), 1115 (s), 1095 (s), 1075 (s), 1035 (m), 975 (m), 950 (w), 900 (s), 885 (s), 835 (w), 815 (w), 685 (w), 657 (w).

THE PREPARATION OF SOME THIOSEMICARBAZONES AND THEIR COPPER COMPLEXES

PART II[1]

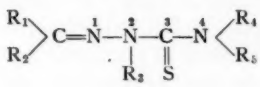
B. A. GINGRAS, R. L. SOMORJAI,[2] AND C. H. BAYLEY

ABSTRACT

Reactions of N- and S-substituted thiosemicarbazones with Cu(I) have been examined. No complexes are formed when the S atom is substituted and(or) when the N^2 hydrogen atom is replaced by Me. 4,4-Dimethyl derivatives not only give the usual 1:1 complexes, but also 2:1 complexes (2 thiosemicarbazones to 1 copper) in which copper has been oxidized to Cu(II). Reactions of thiosemicarbazones with Cu(II) give 1:1, 2:1, and probably a mixture which corresponds to 2:3 complexes. A discussion on the possible structures for the 1:1 complexes is given and they are best represented as polymers, the metal being bonded to the sulphur and to a nitrogen atom of the thiosemicarbazide portion of the molecule. Infrared spectra of these compounds have been examined, particularly in the 1100 cm^{-1} region where a strong to medium band disappears in going from thiosemicarbazones to copper complexes. It is suggested that this band is due to the C=S vibration.

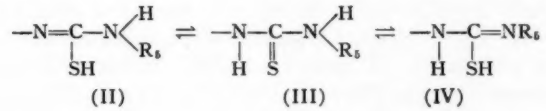
Various metallic complexes of organic sulphur compounds are described in the literature (1–10). Many of these were prepared for physiological studies and their structures are not discussed fully (7, 10). In earlier publications we have reported the preparation of Cu(I) complexes of thiosemicarbazones* (1 tsc:1 Cu) (11) and their fungicidal action (12). This paper presents a discussion of the possible structures for these complexes based mainly on the reactions of several *N*-methyl thiosemicarbazones with copper ions in ammoniacal and neutral media.

Thiosemicarbazones have the structure (I) (R = alkyl, aryl, or H).

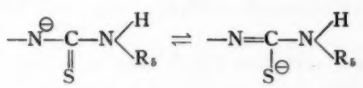


(I)

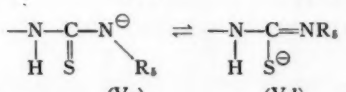
When R_3 and R_4 and(or) R_5 are hydrogen atoms, tautomeric thiols (II) and (IV) and thione (III) forms are possible, these being interconvertible through their common anions (V) and cations (VI). The dipolar structures (III*a*) and (III*b*) may contribute to (III).



(II) (III) (IV)



(V*a*) (V*b*)



(V*c*) (V*d*)

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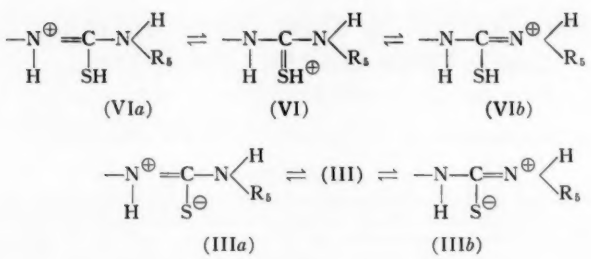
* *tscs.*



(VI*a*) (VI) (VI*b*)

(III*a*) (III*b*)

Infrared absorption spectra of tscs in the solid state indicate the absence of tautomerism, the thione form (III) being the exclusive structure (13). On the other hand most tscs dissolve in alkali (13, 14) and form metallic complexes in alkaline solution (5, 8, 11) thus suggesting the important role of one of the anions (V) in solution. The observation that N^2-methyl tscs (I, R_3 = Me) do not dissolve in alkali (13) and that hydrazides of type (R—CO—NH—NRR) do not form copper complexes if the H atom is replaced by Me (15), suggest that the N^2 hydrogen atom is essential for the complex formation in these compounds. Apparently the resonance structure (III*a*) and consequently anion (V*b*) are the main contributing forms in alkaline solutions. This is plausible because of the greater stability conferred on anion (V*b*) through the formation of a conjugated unit, C=N—N=C, where the delocalization of the π electrons lowers the free energy of the system. Anion (V*a*) is probably less important as a contributing form partly because of the lack of the above-stated conjugation and partly because the sulphur atom has more available orbitals to accommodate extra electrons than the nitrogen atom (16). These observations agree with a number of published data. Wilson and Burns (14) found that the sodium salt of acetone tsc had retained the terminal NH_2 group. Jensen *et al.* prepared several substituted thiosemicarbazides (4, 5, 6) and found that neither in alkaline nor in acidic media did S-substituted thiosemicarbazides form complexes (6), indicating the necessity of a metal–sulphur bonding. Jensen also concluded that co-ordination in M(II)-thiosemicarbazide and M(II)-semicarbazide complexes is through the N^1 atom (4, 5, 6, 17). He arrived at this conclusion from the observation that 1-aryl thiosemicarbazide, ($ArN^1H—N^2H—C^3S—N^4H_2$), did not form Ni(II) complexes, and 1-acyl ($RCON^1H—N^2H—C^3S—N^4H_2$), 1,1-disubstituted thiosemicarbazides ($R_1R_2N^1—N^2H—C^3S—N^4H_2$) as well as tscs (I) gave no Cu(II) complexes in acidic media (6).

The fact that thiobenzhydrazide (Ph—CS—$NHNH_2$) forms a Ni(II) complex similar to the corresponding thiosemicarbazide complex, supports the theory of co-ordination through the N^1 atom (1).

In Part I (11) it has been found that Cu(I) ions in ammoniacal solution formed 1:1 complexes with tscs, one hydrogen atom being presumably replaced by Cu. We have now prepared a series of *N*-methyl and S-substituted tscs (Table I) and examined their reaction with Cu(I) ions in ammoniacal solution (Table II) and with Cu(II) ions in alcoholic solution (Table III).

With Cu(I) ions, complexes were formed in all instances, except with the N^2-methyl (I, R_3 = Me) and *S*-methyl tscs. These various reactions are summarized in Table II. These observations support the conclusions concerning the importance of anion (V*b*) (N^2-hydrogen being replaced) and the S–metal linkage.

TABLE I

Substituted thiosemicarbazones

No.	Name	m.p., °C	Yield, %	Solvent used in recryst.	Formula	Found, %	Calc., %	I.R. bands in cm^{-1}*			Reference for preparation of substituted thiosemicarbazides
								3500–3000	1630–1515	1130–1010	
1	Benzaldehyde 2-methyl tsc	175	78	EtOH	$C_9H_{11}N_3S$	C, 56.19 H, 5.78 N, 21.50 S, 16.35	C, 56.00 H, 5.70 N, 21.72 S, 16.58	3460 m 3280 m 3130 w	1582 s	1083 m 1050 w 1010 s	28
2	Benzaldehyde 4-methyl tsc	163	93	EtOH–C_6H_{14}	$C_9H_{11}N_3S$	C, 55.57 H, 6.30 N, 21.90 S, 16.40	C, 56.00 H, 5.70 N, 21.72 S, 16.58	3325 m 3150 m	1550 b	1090 m 1070 m 1035 s	29
3	Benzaldehyde 2,4-dimethyl tsc	117.5	100	EtOH–C_6H_{14}	$C_{10}H_{13}N_3S$	C, 58.19 H, 6.21 N, 20.40 S, 15.30	C, 57.97 H, 6.28 N, 20.29 S, 15.46	3370 m	1605 w 1580 w 1530 s	1060 s 1050 w	30
4	Benzaldehyde *S*,4-dimethyl tsc	66	55	C_6H_{14}	$C_{10}H_{14}N_3S$	C, 58.10 H, 6.19 N, 20.20 S, 15.30	C, 57.80 H, 6.73 N, 20.20 S, 15.40	3290 m	1615 s 1583 s	1085 m 1055 w 1035 m	
5	Benzaldehyde 4,4-dimethyl tsc	148 s	77	MeOH	$C_{10}H_{13}N_3S$	C, 57.98 H, 6.45 N, 20.20 S, 15.08	C, 57.97 H, 6.28 N, 20.29 S, 15.46	3260 w 3180 m	1610 m 1565 s	1080 m 1065 m 1030 m	31
6	*p*-Chlorobenzaldehyde-2,4-dimethyl tsc	146 s	100	MeOH–acetone	$C_{10}H_{12}ClN_3S$	C, 49.64 H, 5.08 Cl, 14.95 N, 17.35 S, 13.34	C, 49.68 H, 4.97 Cl, 14.72 N, 17.39 S, 13.24	3340 m	1605 m 1595 m	1093 v 1060 s 1040 w 1015 w	30
7	*p*-Chlorobenzaldehyde-4,4-dimethyl tsc	132	96	*n*-Hexane	$C_{10}H_{12}ClN_3S$	C, 50.56 H, 4.51 Cl, 14.46 N, 17.18 S, 13.00	C, 49.68 H, 4.97 Cl, 14.72 N, 17.39 S, 13.24	3120 m	1595 m 1515 m	1090 m 1060 m 1015 m	31
8	Heptanal-2-methyl tsc	52	40	*n*-Hexane	$C_9H_{19}N_3S$	C, 53.45 H, 9.13 N, 21.35 S, 16.44	C, 53.7 H, 9.45 N, 20.85 S, 15.9	3420 m 3260 m 3120 m	1585 m	1085 vw 1040 w	28

TABLE I

Substituted thiosemicarbazones (*concluded*)

No.	Name	m.p., °C	Yield, %	Solvent used in recryst.	Formula	Found, %	Calc., %	I.R. bands in cm^{-1}*			Reference for preparation of substituted thiosemicarbazides
								3500–3000	1630–1515	1130–1010	
9	Octanal-2-methyl tsc	45	35	*n*-Hexane†	$C_{10}H_{21}N_3S$	C, 56.57 H, 8.82 N, 19.61 S, 14.85	C, 55.9 H, 9.76 N, 19.54 S, 14.92	3190 vw 3120 w	1592 s 1505 s	1040 m 1030 m 1007 w	28
10	Octanal-4-methyl tsc	51	47	*n*-Hexane	$C_{10}H_{21}N_3S$	C, 55.85 H, 10.32 N, 19.55 S, 14.60	C, 55.9 H, 9.76 N, 19.54 S, 14.92	3280 m 3180 m	1560 b 1535 w	1080 m 1045 m 1025 w	29
11	Nonanal-2-methyl tsc	62.5	73	*n*-Hexane	$C_{11}H_{23}N_3S$	C, 57.59 H, 9.86 N, 18.41 S, 14.02	C, 57.61 H, 10.12 N, 18.32 S, 13.95	3400 m 3200 m 3100 m	1585 s	1055 sh 1035 w	28
12	Decanal-2-methyl tsc	45	64	*n*-Hexane	$C_{12}H_{25}N_3S$	C, 59.60 H, 10.30 N, 16.95 S, 13.31	C, 59.25 H, 10.37 N, 17.28 S, 13.16	3420 m 3250 m 3120 w	1585 m	1050 m	28
13	Undecanal-2-methyl tsc	73.5	75	EtOH	$C_{13}H_{27}N_3S$	C, 60.53 H, 10.43 N, 16.25 S, 12.86	C, 60.7 H, 10.5 N, 16.3 S, 12.45	3420 m 3250 m 3120 m	1588 s (Nujol)	1090 w 1068 w 1050 w 1025 vw	28
14	9-Undecenal-2-methyl tsc	33	20	*n*-Hexane	$C_{13}H_{25}N_3S$	C, 61.57 H, 9.64 N, 16.65 S, 12.60	C, 61.25 H, 9.8 N, 16.45 S, 12.50	3460 m 3300 m 3140 m	1580 b	1075 w	28
15	Acetone-*S*-benzyl tsc	50	50	*n*-Hexane	$C_{11}H_{15}N_3S$	C, 59.70 H, 6.90 N, 18.95 S, 14.30	C, 59.68 H, 6.84 N, 19.00 S, 14.47	3500 m 3300 m 3130 m	1545 m 1505 m	1075 m 1035 m	14

*From KBr pellets.
†After purification by chromatography.

TABLE II

Reactions between methyl derivatives of tscs and Cu(I) in ammoniacal solution

tsc No. from Table I	Type of complex formed	m.p., °C decomp.	Yield, %	Formula	Found, %	Calc., %	I.R. bands in cm^{-1}*		
							3500–3000	1630–1515	1130–1010
1	None								
2	1:1	207–208.5	85	$C_9H_{10}N_3SCu$	C, 42.23 H, 4.00 N, 16.20 S, 11.99 Cu, 25.2	C, 42.30 H, 3.91 N, 16.42 S, 12.6 Cu, 24.85	3380 s 3060 vw 3030 vw	1580 m 1550 b	1065 s 1013 m
3	None								
4	None								
5	(*a*) 1:1	225	25	$C_{10}H_{12}N_3SCu$	C, 43.73 H, 3.69 N, 14.81 S, 11.38 Cu, 23.4	C, 44.57 H, 4.49 N, 15.58 S, 11.87 Cu, 23.55	3060 vw	1600 m 1575 m	1080 w 1035 m
	(*b*) 2:1	185–187	40	$C_{20}H_{24}N_6S_2Cu$	C, 50.50 H, 5.80 N, 17.59 S, 13.18 Cu, 13.33	C, 50.42 H, 5.08 N, 17.64 S, 13.44 Cu, 13.33	3070 vw	1590 w 1570 m 1510 s	1065 m 1040 m
6	None								
7	2:1	232	60	$C_{20}H_{22}Cl_2N_6S_2Cu$	C, 43.66 H, 3.88 Cl, 13.15 N, 15.18 S, 11.69 Cu, 12.26	C, 44.00 H, 4.25 Cl, 13.02 N, 15.40 S, 11.68 Cu, 11.65	3120 vw	1595 m 1560 w 1515 s	1085 m 1060 w 1025 m
8	None								
9	None								
10	Not attempted								
11	None								
12	None								
13	None								
14	Not attempted								
15	Unidentified compound								

*From KBr pellets.

TABLE III

Reaction of tscs with Cu(II) in alcoholic solution

tscs	Type of complex	m.p., °C decomp.	Yield, %	Formula	Found, %	Calc., %	Remarks
Benzaldehyde tsc	1:1	184–185	49	$C_8H_8N_2SCu$			Identical with the one described in Part I (11)
Benzaldehyde-4,4-dimethyl tsc	1:1	225	40	$C_{10}H_{12}N_2SCu$			Identical with the one described in Table II
	2:1	185	40	$C_{20}H_{24}N_6S_2Cu$			Identical with the one described in Table II
o-Chlorobenzaldehyde tsc	1:1	198	52	$C_8H_7ClN_2SCu$			Identical with the one described in Part I (11)
m-Fluorobenzaldehyde tsc	1:1	162	58	$C_8H_7FN_2SCu$			Identical with the one described in Part I (11)
n-Octanal tsc	2:1	122	Trace	$C_{18}H_{37}N_6S_2Cu$	Cu, 15.4	Cu, 13.75	Not enough material for further purification and complete analysis
n-Nonanal tsc	2:3	135–138	42	$C_{20}H_{40}N_6S_2Cu_2$	C, 37.27 H, 5.73 N, 13.35 S, 10.45 Cu, 31.0	C, 38.85 H, 6.48 N, 13.58 S, 10.34 Cu, 30.8	
Undecanal tsc	1:1						Identical with the one described in Part I (11)
9-Undecenal tsc	2:3	134–137	58		Cu, 30.7	Cu, 28.9	
Laurylaldehyde tsc	2:1	125	18	$C_{26}H_{52}N_6S_2Cu$	C, 55.17 H, 9.33 N, 14.71 S, 10.75 Cu, 11.66	C, 54.23 H, 9.12 N, 14.60 S, 11.11 Cu, 11.03	
	2:3	137–140	52.5	$C_{26}H_{52}N_6S_2Cu_2$	N, 12.25 S, 9.60 Cu, 27.8	N, 11.95 S, 9.12 Cu, 27.1	

Magnetic moment measurements on two typical complexes indicated zero magnetic moment, and thus the presence of monovalent copper.

Crystal field theory shows that d^{10} ions may co-ordinate octahedrally (sp^3d^2), tetrahedrally (sp^3), or linearly (sp), depending on the nature of the surrounding field (18). Although linear co-ordination occurs in cuprite (19), cuprous chromite (20), and cuprous ferrite (21), only one example of this type of co-ordination with an organic ligand is well-known (e.g. diazoaminobenzene-Cu(I) (22).

Since most Cu(I) ions co-ordinate tetrahedrally (23) and in many cases, the resulting complexes were found to be polymers (24), it is possible that in the case of tsc copper complexes, the metal is bonded to a sulphur and an N^1 atom and co-ordinated further tetrahedrally to a sulphur and N atom of another molecule in a polymeric chain.

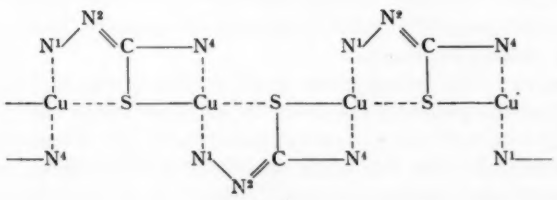
Experimental results obtained with 4,4-dimethyl derivatives of tscs indicate that N^4 nitrogen is probably involved in this co-ordination.

It was found that 4,4-dimethyl tscs form mostly 2:1 complexes when they react in the usual manner with Cu(I). Magnetic moment measurements on (benzaldehyde-4,4-dimethyl tsc)$_2$-copper showed one unpaired electron indicating oxidation of the Cu(I) ion to the Cu(II) state. This change was brought about by an extra Me group on the N^4 atom, since 4-methyl tscs behave normally giving 1:1 complexes only. These observations are difficult to explain unless the Cu ion is actually co-ordinated to the N^4 atom. Oxidation of the metal could have happened by disproportionation (25) as follows:

$$2\text{Cu(I)} \rightarrow \text{Cu(II)} + \text{Cu(0)}.$$

Material balance of starting materials and products in the case of benzaldehyde-4,4-dimethyl tsc supports this view.

Molecular models of various polymeric structures were examined and a few were discarded because of steric factors and(or) too much strain particularly in small rings. Two possible structures are (VII) and (VIII) below.

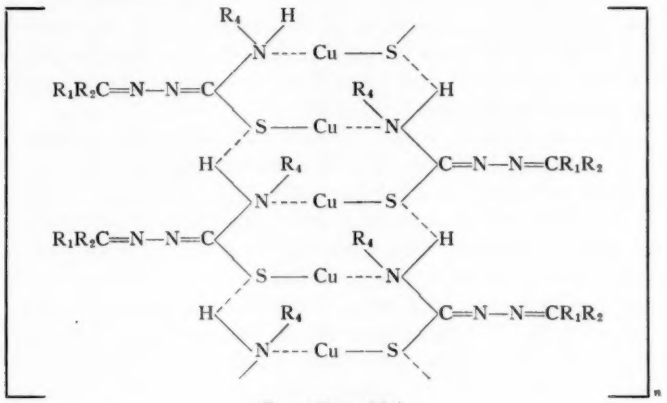


(VII)

Structure (VII) comprises a series of 5- and 4-membered rings in which Cu(I) is co-ordinated tetrahedrally to a sulphur and N^1 atoms of a tsc and a sulphur and N^4 atoms of another molecule. Molecular model of this structure shows a certain amount of strain in the 4-membered ring. Although such a structure accounts for many of the experimental facts, it does not explain easily the anomalous behavior of 4,4-dimethyl tscs.

Structure (VIII) in which copper is linearly co-ordinated to N^4 and sulphur atoms is even more likely and is completely strainless. Molecular models show that the copper atoms lie in a straight line very close to each other suggesting that there may be bonds between them. This would contribute to the insolubility of the complexes as pointed

out by Frasson *et al.** Structure (VIII) accounts for all the facts including the reaction of 4,4-dimethyl tscs and is to be favored.



(R_4 = H or Me)

(VIII)

Several attempts to determine the molecular weight of these complexes failed, but a polymeric structure would conveniently explain their stability and their high insolubility.

Table I lists the various derivatives of tscs prepared and examined in the present work. They were made by the usual method (11) using an aldehyde and the appropriate substituted thiosemicarbazide. The reactions proceeded as expected in all cases except with 2-methyl-*S*-methyl thiosemicarbazide which did not give the corresponding 2,*S*-dimethyl tscs. Instead, benzaldehyde and *p*-chlorobenzaldehyde when treated with that reagent gave the corresponding 2-methyl semicarbazones, the S—Me group being obviously replaced by an oxygen atom. The reaction proceeded normally, however, in the case of *S*-methyl-4-methyl thiosemicarbazide.

Table II is a summary of the reactions of these substituted tscs with Cu(I) ions in alkaline solution, and the products obtained. In all cases where the N^2-hydrogen atom has been replaced by Me, there were no complexes formed. Only about 50% of the original tsc could be recovered as there was some decomposition. Likewise, when the sulphur atom was substituted with a methyl or benzyl group, there were no complexes formed. The fact that, with all other derivatives complexes of some kind were obtained, emphasizes the importance of the N^2-hydrogen atom, and the S—Cu bond and thus the significance of anion (V*b*).

We have also examined the reaction of various tscs with Cu(II) acetate in alcoholic solution (see Table III). The results were quite surprising since, instead of obtaining the expected 2:1 complexes, aromatic tscs gave 1:1 complexes identical with those obtained using Cu(I) in ammoniacal solution. Benzaldehyde 4,4-dimethyl tsc gave a mixture of 2:1 and 1:1 complexes identical with those obtained using Cu(I) as the reagent. Aliphatic tscs gave an insoluble compound which analyzed fairly close to a 2 tsc

**Reference added in proof: E. Frasson, R. Zannetti, R. Bardi, S. Bezzi, and G. Giacometti. The chemistry of the co-ordinate compounds, a symposium. Edited by H. Taube and A. G. Maddock. Pergamon Press. 1958. p. 462.*

to 3 Cu, and in some cases, in addition, the usual 1:1 complexes. The yields were, in every case, much lower than with Cu(I). Kuhn and Zilliken (8) had also found that aromatic tscs gave the same complexes both with Cu(I) and Cu(II), the yield being lower with the latter. It is likely that tscs reduce part of the Cu(II) to Cu(I) which then reacts with the unoxidized tscs. This type of reduction is quite common with organic sulphur compounds containing an oxidizable sulphur (2, 6, 8, 26, 27).

$$2R—S^- + 2Cu^{++} \rightarrow R—S—S—R + 2Cu^+$$

In some cases the disulphides and their copper complexes were obtained (2, 26). We did not isolate the disulphide–Cu complexes but we have identified from the soluble part of the reaction mixture some 2:1 complexes.

Infrared spectra of all these compounds have been taken in Nujol (Part I) and(or) KBr (Tables I, II, and IV). Table IV lists the typical bands in three regions, obtained from KBr disks, of a few tscs and their corresponding Cu(I) complexes. Contrary to the results reported in Part I from infrared spectra taken in Nujol mulls, it can be seen that both classes of compounds show three bands in the N—H stretching region. It appears that the very strong absorption of Nujol overlaps with the band at around 3100 cm^{-1} in certain cases. The two bands at about 3400 and 3200 cm^{-1} are shifted to higher frequencies by about 70 cm^{-1} in the complexes and the third band at 3150 cm^{-1} remains constant or is shifted down by 50 to 70 cm^{-1}. It is known that if a metal co-ordinate bond is formed with a nitrogen atom already bonded to H atoms, a shift of the N—H stretching bands to lower frequencies occurs, probably due to the increase in the polarity of the N—H bond (32). Although the shift to higher frequencies is difficult to explain, there is no doubt that the electronic environment of the NH_2 group has changed.

By examination of the spectra of various methyl-substituted tscs (Table I), it can be seen that differentiation between those three bands is difficult. The N^2-methyl compounds, although they have one less N—H group than free tscs, show the same number of bands in this region. The 2,4-dimethyl derivatives show only one band at around 3370–3340 cm^{-1} while the 4,4-dimethyl derivative of benzaldehyde tsc, with only one N—H group in the molecule shows two bands at 3260 and 3180 cm^{-1}. 4-Methyl compounds have two bands in this region.

The complexes in general show one additional band in the 1600 cm^{-1} region which can be attributed to an extra —C=N— as would be expected from any of the structures discussed above.

Tables I, II, and IV, also contain the region 1130–1010 cm^{-1} where a tentative assignment for the C=S vibration can be made. This vibration has been studied widely with many types of thio compounds and its assignment varies from about 1500 to 800 cm^{-1}. Davies and Jones (33), from a study of thioformamide, point out the difficulty of assigning a characteristic frequency for the C=S. The range 1400–1300 cm^{-1} has been suggested (34), and in thioacetamide the C=S was assigned at 1302 cm^{-1} (35). Lieber *et al.* (36) suggest the region 1395–1346 cm^{-1} in 4-substituted thiosemicarbazides. The band at 1100 cm^{-1} was assigned to C=S in thiopeptides (37), while the region 1200–1000 cm^{-1} was suggested for this vibration in thiocarbonyl compounds (38), and the region 1000 cm^{-1} in secondary thioamides (39). More recently, Bellamy (40) suggested the band at 1080 cm^{-1} in cyclic trithiocarbonates, 1200 cm^{-1} in dithioesters, and near 1000 cm^{-1} in some thioamides.

By comparing the spectra of tscs and their copper complexes (Tables I, II, and IV), it can be seen that a band disappears in the 1130–1080 cm^{-1} region. It is reasonable to

TABLE IV

Infrared absorptions in three regions of some typical tscs and their 1:1 copper complexes*

Compounds	tscs			1:1 copper complexes		
	3500–3000 cm^{-1}	1630–1515 cm^{-1}	1130–1010 cm^{-1}	3500–3000 cm^{-1}	1630–1515 cm^{-1}	1130–1010 cm^{-1}
Acetone	3400 m 3220 m 3150 m	1600 s	1115 m 1085 w 1035 m	3470 m 3300 m 3160 m	1625 m 1600 m	1080 w 1015 m
o-Fluorobenzaldehyde	3440 m 3250 m 3140 m	1600 s	1090 m 1070 m 1040 m	3460 m 3340 m 3140 vw	1620 m 1580 b	1040 w 1025 m
m-Fluorobenzaldehyde	3390 m 3230 m 3140 m	1600 s 1580 w	1100 m 1070 m	3460 m 3360 m 3070 w	1610 m 1575 b	1025 m
o-Chlorobenzaldehyde	3420 m 3250 m 3150 m	1600 m	1080 m 1055 m 1040 m	3490 m 3360 m 3150 vw	1600–1550 b	1060 m 1035 m
2,4-Dichlorobenzaldehyde	3480 m 3280 m 3160 m	1600 s	1120 m 1105 m 1060 m	3460 m 3380 m 3160 m	1600–1550 b	1110 m 1060 m
Nonanal	3400 m 3200–3100 b	1600 s	1110 s 1050 m	3450 m 3270 m 3100 m	1635 s 1610 m	1030 m
Octanal	3400 m 3250 m 3150 m	1600 s	1110 s 1050 m	3450 m 3280 m 3100 m	1610 w 1580 m	1030 m
Undecanal	3440 m 3300 m 3180 m	1610 s	1130 s 1050 m 1015 m	3450 m 3280 m 3100 m	1630 w 1610 m	1030 m

*From KBr pellets.

assume that this band is due to the C=S of the free tsc. Yamaguchi *et al.* (9) found that a strong band at 1083 cm^{-1} in thiourea is extremely weakened or disappears upon complex formation with a metal and they attributed this band to C=S.

In the region where the "thioureide" band has been assigned by Randall (41) between 1613 and 1471 cm^{-1}, Ettlinger (42) at 1508 cm^{-1} and at 1495 cm^{-1} (43), there are no noticeable differences between spectra of tscs and their copper complexes.

EXPERIMENTAL

Thiosemicarbazones were generally prepared by the method of Sah and Daniels (44). Preparations of the various substituted thiosemicarbazides were carried out according to the procedures referred to in Table I, except for *S*-methyl-4-methyl thiosemicarbazide, which is described below. Reactions of substituted tscs with Cu(I) (Table II) were carried out as described in Part I (11). Melting points (corrected), yields, solvents used in purification, bands in three regions of the infrared spectrum, and analyses are shown in the tables.

Preparation of S-Methyl-4-methyl Thiosemicarbazide.HI

A mixture of 4-methyl thiosemicarbazide (32) (14.3 g, 0.1 mole) and methyl iodide (20.5 g, 0.15 mole) dissolved in isopropyl alcohol (115 ml) and acetone (200 ml) was refluxed for 1.5 hours. The solution became yellow and after cooling, the white solid that precipitated out was collected by filtration and recrystallized from absolute alcohol. It had m.p. 164° C; yield: 15 g, 59%. The compound is sensitive to moisture and was kept in dry alcohol and recovered only before use in the next step.

Preparation of Benzaldehyde-S,4-dimethyl tsc (No. 4, Table I)

S,4-Dimethyl thiosemicarbazide.HI (4.94 g, 0.02 mole, corresponding to 2.38 g, 0.02 mole free base) was dissolved in water (60 ml) and neutralized with NaOH *N*/1 (20 ml). The solution turned dark yellow probably due to some liberated iodine. Two drops of acetic acid were added followed by 50 ml of an alcoholic solution of benzaldehyde (2.12 g, 0.02 mole). The clear solution obtained was heated for 15 minutes on the steam bath and cooled. The dark red oil that separated was taken up in ether and dried over drierite. After evaporation of the solvent, the residue was dissolved in *n*-hexane and after cooling, benzaldehyde-*S*,4-dimethyl tsc crystallized out.

Reaction between Benzaldehyde-4,4-dimethyl tsc and CuCl

To a boiling solution of benzaldehyde-4,4-dimethyl tsc (2.4 g, 0.11 mole) in MeOH (50 ml) was added, dropwise, a solution of CuCl (1.16 g, 0.11 mole) in ammonia (25 ml, sp. gr. 0.9). A brown precipitate was formed and heating and stirring were continued for 15 minutes after the addition had been completed. The mixture was allowed to cool and the precipitate was collected by filtration and washed with HCl 3% and water. Part of this solid was soluble in acetone from which it was recrystallized; this was the 2:1 complex (No. 5*b*, Table II). The insoluble fraction was washed successively with ammonia, water, HCl 3%, water, acetone, ether, and alcohol, after which it gave fairly good analysis for the 1:1 complex (No. 5*a*, Table II).

Reaction between p-Chlorobenzaldehyde-4,4-dimethyl tsc and CuCl

This was carried out as above except that the precipitate obtained was not too soluble in acetone. It was dissolved in toluene from which it was recovered by evaporation. This corresponds to the 2:1 complex (No. 7, Table II). No 1:1 complex was isolated in this case from the insoluble residue.

Attempt to React Benzaldehyde-2-methyl tsc with CuCl

The following is typical of attempts made to react 2-methyl-substituted tscs with CuCl.

To a refluxing solution of benzaldehyde-2-methyl tsc (5.8 g, 0.03 mole) in MeOH (200 ml) was added, dropwise, a solution of CuCl (4 g, 0.04 mole) in ammonia (50 ml). A small amount of a precipitate was observed and this when collected was found to be inorganic and had a high copper content (50%). The remaining solution was neutralized with HCl, diluted with water, and cooled. The starting material (3 g) crystallized out; m.p. and mixed m.p. 175° C.

Reaction between p-Chlorobenzaldehyde tsc and Copper Acetate

To a boiling solution of *p*-chlorobenzaldehyde tsc (5.3 g, 0.025 mole) in ethanol (200 ml) was added slowly a solution of cupric acetate (5 g, 0.025 mole) in the same solvent (150 ml). A light yellow compound precipitated out and, after cooling, was collected by filtration and washed on the filter with hot acetone. This precipitate was found to be the 1:1 complex, identical with the one described in Part I; m.p., mixed m.p., and infrared spectra. No other products were isolated from the mother liquor.

Other reactions with cupric acetate were carried out in a similar manner and the various products obtained are described in Table III.

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THE INTERACTION OF BORON TRIFLUORIDE WITH HYDRAZINE[1]

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ABSTRACT

Boron trifluoride and anhydrous hydrazine react rapidly at 25° *in vacuo* yielding a 1:1 complex compound, $BF_3 \cdot N_2H_4$ (m.p. 87°), while at higher temperatures, or with diethyl ether as solvent, they form complex mixtures containing 52.2 to 63.5 mole percent BF_3; but in tetrahydrofuran solution the 2:1 adduct, $2BF_3 \cdot N_2H_4$ (m.p. 260°) is formed. The X-ray powder diffraction pattern of $BF_3 \cdot N_2H_4$ consists of 28 lines from which a unit cell with triclinic symmetry was calculated. The thermal decomposition of $BF_3 \cdot N_2H_4$ is complex, yielding in part N_2, NH_3, NH_4BF_4, and BN. Hydrolysis of $BF_3 \cdot N_2H_4$ yields a mixture of hydroxyfluoroborate ions. At −80°, $BF_3 \cdot N_2H_4$ absorbs NH_3 to form a diammoniate which liberates NH_3 slowly at higher temperatures. Partial displacement of BF_3 from $BF_3 \cdot N_2H_4$ by HCl and partial absorption of HCl by $BF_3 \cdot N_2H_4$ occur simultaneously at 110°.

Infrared spectra of $BF_3 \cdot N_2H_4$ and $2BF_3 \cdot N_2H_4$ have been measured in the range of 4000 to 650 cm^{-1} and frequency assignments have been made.

Boron trifluoride forms co-ordination complexes with a variety of nitrogen compounds such as ammonia, amines, imines, amides, nitriles, and nitrogen-containing heterocyclics (1, 2, 3). The lone electron pair on the nitrogen atom in these compounds readily forms a co-ordinate bond with the electron-deficient boron trifluoride molecule. Since hydrazine has two available electron pairs, one on each nitrogen atom, it is interesting to determine whether this molecule will co-ordinate with one or two molecules of boron trifluoride. In two independent studies (4, 5) it was found that diborane reacted with hydrazine to form the addition compound $H_3B \cdot NH_2NH_2 \cdot BH_3$, indicating that both nitrogen atoms in hydrazine are capable of co-ordinating with borane groups. This paper is the first of a series dealing with the reactions of electron-deficient molecules with hydrazine. Since boron trifluoride is one of the most active electron acceptors, its reaction with hydrazine was investigated first.

RESULTS AND DISCUSSION

It was found that the reaction of boron trifluoride with anhydrous hydrazine at −80°, in the absence of a solvent, was extremely slow. At room temperature, however, a solid white 1:1 complex compound, boron trifluoride-hydrazine, $BF_3 \cdot N_2H_4$ (m.p. 87°), was formed within a few minutes. With reaction times of 2 and 3 hours, complexes having ratios of boron trifluoride to hydrazine of 1.09 and 1.16, respectively, were obtained. The latter complex, when treated with additional boron trifluoride in diethyl ether as solvent for 11 hours, gave a substance with an empirical composition of $(BF_3)_{1.72}N_2H_4$. The reaction of boron trifluoride with hydrazine in diethyl ether produced complexes of different stoichiometry. Depending upon such experimental conditions as efficiency of mixing, reaction time, and temperature, substances were obtained with boron trifluoride to hydrazine ratios in the range 1.42 to 1.88, but the ratio was never 2.* Similar results were obtained for the reaction of boron trifluoride with hydrazine at 140 and 200°. With tetrahydrofuran as solvent, however, the product was 2-boron trifluoride-hydrazine, $2BF_3 \cdot N_2H_4$ (m.p. 260°). It is noteworthy that tetrahydrofuran also promoted the for-

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*One of the referees has suggested that the non-stoichiometric mixtures were possibly solid solutions.

mation of 2-boron trifluoride-ethylenediammine (6), whereas the 1:1 adduct resulted in diethyl ether solution.

The formation of 1:1 and 2:1 addition compounds between boron trifluoride and hydrazine indicates that both nitrogen atoms in hydrazine are strong electron-pair donors. This is expected on the basis of the configuration of the hydrazine molecule (7), which shows that the electron pairs on each of the two nitrogen atoms are probably located in tetrahedral (sp^3) hybrid orbitals and are readily accessible for the formation of one or two co-ordinate bonds with suitable acceptor molecules. While the 1:1 adduct formed easily by direct interaction of boron trifluoride with hydrazine, the 2:1 adduct could not be obtained in the same way. Apparently the formation of one co-ordinate link reduces the availability of the lone electron pair on the other nitrogen atom for bonding with a second boron trifluoride molecule. The decreased reactivity of the second electron pair in hydrazine seems to be a general rule (8). In tetrahydrofuran solution both electron pairs appear to be readily available so that the 2:1 complex was the only product.

The hydrazine–boron trifluoride phase diagram in the range 46.5 to 66.7 mole% boron trifluoride (Fig. 1) clearly shows a maximum at 50 mole% boron trifluoride corresponding

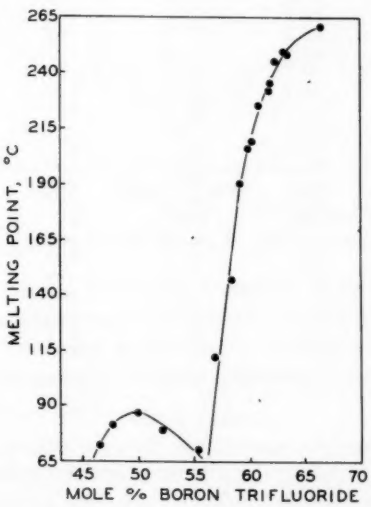

FIG. 1. A portion of the phase diagram of the system hydrazine – boron trifluoride.

to the 1:1 adduct. A second maximum at 66.7 mole% boron trifluoride, corresponding to the 2:1 adduct, is inferred since at the extreme right of the complete phase diagram, i.e. at 100 mole% boron trifluoride, the curve must drop to −127°, the melting point of pure boron trifluoride. The absence of a peritectic point in the region investigated rules out the possibility of incongruently melting compounds of intermediate stoichiometry, such as, $3BF_3 \cdot 2N_2H_4$. The phase diagram was investigated only in the range 46.5 to 66.7 mole% boron trifluoride because it was not possible to obtain, by direct interaction or by reaction in a solvent, solid complex compounds with boron trifluoride contents outside this range.

The compound boron trifluoride-hydrazine was insoluble in diethyl ether and slightly

soluble in acetone. The compound 2-boron trifluoride-hydrazine was soluble in tetrahydrofuran. Both new compounds were hygroscopic and dissolved readily in water yielding acidic solutions, which retained the reducing properties of hydrazine in that these aqueous solutions decolorized instantly an aqueous solution of potassium permanganate.

Infrared spectra of the 1:1 and 2:1 complex compounds (Fig. 2), obtained by the

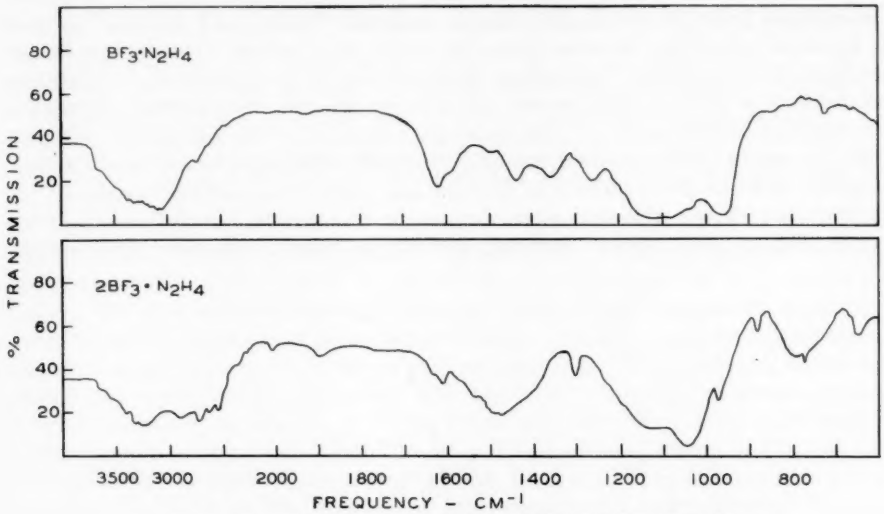

FIG. 2. Infrared spectra of $BF_3 \cdot N_2H_4$ and $2BF_3 \cdot N_2H_4$ in KBr disks.

potassium bromide pellet technique, consist of broad and unresolved bands which nevertheless indicate vibration frequencies (Table I) characteristic of the constituent BF_3 and N_2H_4 molecules together with a B—N stretching frequency in the vicinity of 1035 cm^{-1}. The assignments in Table I have been made by analogy with the infrared spectra

TABLE I

Infrared absorption bands of $BF_3 \cdot N_2H_4$ and $2BF_3 \cdot N_2H_4$

$BF_3 \cdot N_2H_4$ (cm^{-1})	$2BF_3 \cdot N_2H_4$ (cm^{-1})	Assignment
3650 to 3050, s, b	3650 to 2550, s, b	N—H stretching
1620 s	1610 m	NH_2 deformation (asym.)
1440 s	1475 s, b	NH_2 deformation (sym.)
1355 m	1300 m	NH_2 rocking
1260 m		Unidentified
1100 vs, vb; 1035 s, sh, u	1200 to 1040, s, u	B—F asym. stretching and B—N stretching (BF_4^-?)
965 s	970 s	N—N stretching
	883 w	Unidentified
725 w	775 m	NH_2 wagging and BF_3 bending

s = strong, m = medium, w = weak, b = broad, u = unresolved

of N_2H_4 (7), $BF_3 \cdot NH_3$ (9), $C_5H_5N \cdot BF_3$ (10), $N_2H_4 \cdot HCl$ (11), and $N_2H_4 \cdot 2HCl$ (12). It is not possible to confirm or deny the presence of BF_4^- since its characteristic band at 1020–1100 cm^{-1} (13) might be superimposed on the B—F and B—N stretching frequencies which also occur in this region.

The Debye–Scherrer X-ray powder diffraction pattern of $BF_3 \cdot N_2H_4$ consists of 28 lines, the intensities and interplanar spacings of which are given in Table II. From these

TABLE II

X-ray powder diffraction data for $BF_3 \cdot N_2H_4$*

Line	Intensity	d_{obs} (Å)	d_{calc} (Å)	*hkl*
1	vvw	5.50	5.52	100
2	vvw	4.87	4.87	010
3	vs	4.27	4.23	001
4	vs	3.47	3.47	$\bar{1}01$
5	vw	3.25	3.25	101
6	vw	3.11	3.10	$2\bar{1}0$
7	vvw	2.96	2.99	110
8	s	2.78	2.76	200
9	s	2.43	2.43	011, 020
10	m	2.31	2.31	$3\bar{2}1$
11	vw	2.22	2.24	$1\bar{2}2$
12	w	2.11	2.11	002
13	w	2.03	2.03	210, 111
14	w	1.93	1.93	$\bar{1}02$
15	vvw	1.80	1.80	$1\bar{3}0$
16	vvw	1.76	1.76	$\bar{1}21$
17	vvw	1.65	1.65	021
18	w	1.57	1.56	$0\bar{1}2$
19	vvw	1.48	1.49	$0\bar{4}1$
20	vvw	1.44	1.44	$\bar{3}02$
21	vvw	1.39	1.39	130
22	vvw	1.34	1.34	103, 302
23	vvw	1.26	1.26	$2\bar{1}2$
24	vvw	1.23	1.24	$0\bar{1}4$
25	vvw	1.19	1.19	$4\bar{1}0$
26	vvw	1.16	1.16	$\bar{3}03$
27	vvw	1.13	1.13	$\bar{2}13$
28	vvw	1.11	1.10	500

s = strong, m = medium, w = weak, v = very

*A 57.30-mm camera was used.

measurements the following dimensions of a crystallographic unit cell with triclinic symmetry were calculated by applying Ito's reciprocal lattice method (14): $a = 6.93$ Å, $b = 5.50$ Å, $c = 6.78$ Å; $\alpha = 111.5°$, $\beta = 64.0°$, $\gamma = 88.3°$. While these dimensions represent a solution of the observed X-ray powder pattern, they must be confirmed by X-ray pattern measurements on a single crystal of $BF_3 \cdot N_2H_4$ before they can be accepted as the dimensions of the true unit cell. Other boron trifluoride adducts such as $BF_3 \cdot NH_3$, $BF_3 \cdot CH_3NH_2$, and $BF_3 \cdot (CH_3)_3N$ have orthorhombic, monoclinic, and hexagonal symmetry respectively (15).

Boron trifluoride-hydrazine was stable indefinitely *in vacuo* at room temperature. Decomposition occurred slowly at 190° and was almost complete at about 300° after 5 hours, yielding mainly nitrogen, ammonia, ammonium fluoroborate, and boron nitride. Since the boron–nitrogen bond is probably the weakest one in the addition compound, it seems reasonable to assume that the first step in the pyrolysis was the dissociation reaction,

$$N_2H_4 \cdot BF_3 \rightarrow BF_3 + N_2H_4.$$

Boron trifluoride is stable at 300° (1) while hydrazine decomposes slowly at this temperature into ammonia and nitrogen (16, 17)

$$3N_2H_4 \rightarrow 4NH_3 + N_2.$$

Subsequent interaction of ammonia with boron trifluoride to give a 1:1 adduct,

$$NH_3 + BF_3 \rightarrow NH_3 \cdot BF_3$$

could be followed by its thermal decomposition into ammonium fluoroborate and boron nitride (18),

$$4(NH_3 \cdot BF_3) \rightarrow 3NH_4BF_4 + BN.$$

The over-all reaction, taken as the sum of the above reactions,

$$12(BF_3 \cdot N_2H_4) \rightarrow 4N_2 + 4NH_3 + 9NH_4BF_4 + 3BN$$

accounted quantitatively for the nitrogen production (the ratio of $BF_3 \cdot N_2H_4$ used to N_2 produced was 2.98), but only qualitatively for the other observed products.

The apparent molecular weight of $BF_3 \cdot N_2H_4$ in aqueous solution (49.5 and 54.5 in separate experiments) was approximately one half of the calculated formula weight (99.9), suggesting that the initial action of water gave two ions per formula weight, probably by the following reaction:

$$BF_3 \cdot N_2H_4 + H_2O \rightleftarrows N_2H_5^+ + BF_3OH^-.$$

Since positive tests for fluoroborate ion, BF_4^-, were obtained, it appeared that the hydroxyfluoroborate ion might have been involved in a disproportionation reaction:

$$2BF_3OH^- \rightleftarrows BF_4^- + BF_3(OH)_2^-.$$

Disproportionation of $BF_2(OH)_2^-$, as represented by (19):

$$2BF_2(OH)_2^- \rightleftarrows BF_4^- + B(OH)_4^- \rightleftarrows B(OH)_3 + OH^-$$

was presumed to occur in view of the fact that the basicity of the aqueous solution increased slightly on standing. Although the various ionic species (except BF_4^-) were not identified, the results were consistent with the observation that the hydrolysis of boron trifluoride itself yields a mixture of hydroxyfluoroboric acids (1).

No significant reaction occurred between anhydrous hydrogen chloride and boron trifluoride-hydrazine either at −96° or at 25°, but at 110°, the results shown in Table III were obtained. Three points seem noteworthy: (i) evidently HCl displaced BF_3

TABLE III

Results for the interaction of HCl with $BF_3 \cdot N_2H_4$

Experiment number	1	2
Initial HCl, mmole	8.05	2.73
Initial $BF_3 \cdot N_2H_4$, mmole	3.73	2.71
Reaction time, hr	15	15
Recovered HCl, mmole	4.36	0.60
Consumed HCl, mmole	3.69	2.13
Liberated BF_3, mmole	0.61	0.23
% BF_3 displaced	16.4	8.6

from $BF_3 \cdot N_2H_4$, (ii) the amount of HCl absorbed by the complex was much greater than the amount of BF_3 liberated, suggesting that 1:1:1 complex $BF_3 \cdot N_2H_4 \cdot HCl$ was

formed in substantial quantities, and (iii) the extent of displacement of BF_3 decreased when the initial amount of HCl was lower.

At $-80°$, $BF_3 \cdot N_2H_4$ readily absorbed ammonia, forming a diammoniate, $BF_3 \cdot N_2H_4 \cdot 2NH_3$. On warming to room temperature ammonia was evolved in two stages, but it was not possible to isolate complexes of definite stoichiometry such as $BF_3 \cdot N_2H_4 \cdot NH_3$. Ammonia was thought to be bound to $BF_3 \cdot N_2H_4$ by weak dipole–dipole forces.

EXPERIMENTAL

Since most of the materials used in this work are gaseous and react rapidly with atmospheric moisture, it was necessary to manipulate them in a standard vacuum system (20) equipped with stopcocks and ground-glass joints lubricated with Kel-F grease. Prolonged contact of vapors with this grease was avoided by using all-glass reaction vessels and storage bulbs fitted with capillary break-seals instead of stopcocks. The purity of a volatile compound was checked by determination of its vapor pressure and molecular weight (M). Molecular weights were determined by the vapor density method with an accuracy usually better than 1%. Debye–Scherrer X-ray powder diffraction patterns were obtained from samples sealed in glass tubes of 0.01-mm wall thickness. The pictures were taken on a Seifert apparatus using the Cu $K\alpha$ radiation filtered by nickel. Infrared spectra were measured with a Perkin–Elmer Model 21 double-beam spectrophotometer fitted with sodium chloride optics.

Anhydrous hydrazine was prepared by refluxing the 95+% material (Eastman Kodak) for 3 hours over potassium hydroxide pellets in an atmosphere of dry nitrogen. The fraction boiling in the range 111–113° was found to be 100% pure by the iodate method (8). Commercial boron trifluoride, hydrogen chloride, and ammonia were purified by low-temperature fractionation.

Interaction of Boron Trifluoride with Hydrazine

In a typical experiment boron trifluoride (11.63 mmoles) and anhydrous hydrazine (5.89 mmoles) were brought together *in vacuo* and kept at room temperature for 30 minutes before the mixture was frozen with liquid air. A vigorous exothermic reaction yielded a white solid which was involatile at room temperature and contained boron trifluoride and hydrazine in a 1:1 ratio, corresponding to the compound $BF_3 \cdot N_2H_4$ (found: N_2H_4, 33.3%. $BF_3 \cdot N_2H_4$ requires: N_2H_4, 32.1%). The amount of unchanged boron trifluoride (5.81 mmoles. Found: M, 67.8. Calc. for BF_3: M, 67.8) recovered by distillation at $-80°$, also indicated that boron trifluoride (5.82 mmoles) had reacted with hydrazine (5.89 mmoles) in a 1:1 ratio. The melting point of the adduct was 87°. Using identical procedures and longer reaction times, complex mixtures with BF_3/N_2H_4 ratios of 1.09 and 1.16 were obtained (Table IV). The latter product (0.237 g) was treated with additional

TABLE IV

Boron trifluoride-hydrazine complex mixtures

Composition ratio BF_3/N_2H_4	Reaction conditions	Analysis % N_2H_4
1.09	2 hr *in vacuo* at 25°	30.4
1.16	3 hr *in vacuo* at 25°	28.3
1.40	Diethyl ether solution	25.2
1.70	Diethyl ether solution	21.8
1.84	Diethyl ether solution	20.4
1.88	Diethyl ether solution	20.1

boron trifluoride (15.3 mmoles) in diethyl ether (15 ml) for 11 hours. A substance of empirical composition $(BF_3)_{1.72}\cdot N_2H_4$ (found: N_2H_4, 21.6%) was recovered after distillation of the solvent and excess boron trifluoride at room temperature.

Additional complexes with different compositions, were obtained by the interaction of boron trifluoride and hydrazine in diethyl ether as solvent (Table IV). Similar results were obtained for the reaction *in vacuo* of boron trifluoride with hydrazine above room temperature. In a typical experiment, boron trifluoride (11.7 mmoles) and hydrazine (3.87 mmoles) were sealed in an all-glass reaction vessel and kept for 4 hours at 140°. The amount of boron trifluoride recovered (4.67 mmoles; found: M, 67.5; calc. for BF_3: M, 67.8) by distillation at room temperature showed that the white solid reaction product contained BF_3 and N_2H_4 in a ratio of 1.84:1 (found: N_2H_4, 20.9%; calc. for $(BF_3)_{1.84}\cdot N_2H_4$: N_2H_4, 19.8%).

In another experiment boron trifluoride (34.5 mmoles), hydrazine (10.1 mmoles), and anhydrous tetrahydrofuran (25 g) were condensed together in an all-glass reaction vessel which was then sealed off *in vacuo*. The reactants were stirred (magnetically) overnight at room temperature; a clear solution resulted. The solvent and the excess of boron trifluoride were removed by distillation under low pressure at room temperature; this left a white crystalline deposit of a new compound, 2-boron trifluoride-hydrazine, $2BF_3\cdot N_2H_4$ (found: N_2H_4, 19.25%; calc. for $2BF_3\cdot N_2H_4$: N_2H_4, 19.12%). Its melting point was 260°.

Pyrolysis of $N_2H_4\cdot BF_3$

Vapor pressure measurements in the range 140 to 220° showed that the compound decomposed irreversibly, with nitrogen and ammonia as the only volatile products. Pyrolysis of the adduct (5.65 mmoles) at 275–300° for 4.5 hours in an all-glass vessel gave nitrogen (1.72 mmoles; M: found, 27.8; calc., 28.0) together with a white solid residue which contained some unchanged 1:1 compound (0.52 mmole). An X-ray powder diffraction pattern showed that ammonium fluoroborate and boron nitride were the major components, with the former present in much larger amount. The presence of at least one unidentified solid compound in the residue was suggested by the X-ray data. Although reproducible analytical results were obtained for fluoroborate ion, these were considered unreliable in view of the fact that the hydrolysis of unchanged 1:1 compound yields an uncertain amount of fluoroborate ion.

Hydrolysis of $BF_3\cdot N_2H_4$

Two aqueous freezing point depression measurements indicated apparent molecular weights in solution of 49.5 and 54.5, or approximately one half the formula weight of $BF_3\cdot N_2H_4$, which is 99.9. Potassium chloride (1), cesium sulphate (1), and nitron acetate (21) solutions all gave precipitates when added to an aqueous solution of $BF_3\cdot N_2H_4$, thus confirming the presence of fluoroborate ion, BF_4^-. It was also noted that the initial acidity (pH = 4.35) of a dilute aqueous solution of $BF_3\cdot N_2H_4$ decreased slightly (pH = 4.65) on standing for 35 minutes.

Interaction of $BF_3\cdot N_2H_4$ with Hydrogen Chloride

Anhydrous hydrogen chloride (8.05 mmoles) and boron trifluoride-hydrazine (3.73 mmoles) were sealed in an all-glass vessel and left overnight at 110°. The products of the reaction were a non-volatile solid and a gas which was recovered by distillation at −80° (4.97 mmoles; found: M, 40.3; calc. for HCl: M, 36.5; calc. for BF_3: M, 67.8). The infrared spectrum of the gas showed clearly that it was a mixture of HCl (22) and BF_3 (23).

From the molecular weight value of 40.3, it was calculated, assuming ideal gaseous behavior, that this volatile fraction consisted of 4.36 mmoles HCl and 0.61 mmole BF_3. Thus 3.69 mmoles of HCl reacted with 3.73 mmoles of $BF_3 \cdot N_2H_4$ yielding a solid residue and 0.61 mmoles BF_3. The solid residue was probably a complex mixture of $BF_3 \cdot N_2H_4 \cdot HCl$, $N_2H_4 \cdot HCl$, and $N_2H_4 \cdot 2HCl$, which could not be separated by fractional sublimation.

In an attempt to prepare pure $BF_3 \cdot N_2H_4 \cdot HCl$, $BF_3 \cdot N_2H_4$ (2.71 mmoles) and HCl (2.73 mmoles) were condensed together and kept overnight at 110° in an all-glass reaction vessel. Again the products were a non-volatile solid and a gas (0.835 mmole; found: M, 45.2), which was shown by its infrared spectrum to be a mixture of BF_3 and HCl. The composition of this volatile mixture was estimated to be 0.60 mmole of HCl and 0.23 mmole of BF_3. Thus, as before, it appeared that both displacement of BF_3 from $BF_3 \cdot N_2H_4$ by HCl and absorption of HCl by $BF_3 \cdot N_2H_4$ occurred simultaneously.

Interaction of $BF_3 \cdot N_2H_4$ with Ammonia

When anhydrous ammonia (10.38 mmoles) and $BF_3 \cdot N_2H_4$ (1.86 mmoles) were left in contact for 1 hour at −80°, the amount of ammonia (6.66 mmoles; found: M, 17.0; calc. for NH_3: M, 17.03) recovered by distillation at −80° indicated that the diammoniate, $BF_3 \cdot N_2H_4 \cdot 2NH_3$, had been formed at −80°. Above −80°, this crystalline compound dissociated irreversibly, evolving ammonia in what appeared to be two distinct stages as evident from the results summarized in Table V, but it was not possible to confirm the

TABLE V

Dissociation of the diammoniate of $BF_3 \cdot N_2H_4$

Distillation temperature, °C	Ammonia recovered, mmole	Molecular weight of recovered ammonia (calc. M, 17.03)
−64	0.64	17.1
−46	0.81	17.9
−26	None	—
0	1.50	17.4
+28	0.75	17.2

presence of the monoammoniate. Measurements of the dissociation pressure were not reproducible and the conventional plot of log $p_{(mm)}$ against $1/T$ was always concave to the reference axes.

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ORTHO-SUBSTITUTED CHLOROBENZENES RELATED TO METAMECONINE[1]

J. A. McRae,[2] Marjorie Allen,[3] and R. Y. Moir[4]

ABSTRACT

Chlorinated derivatives of metameconine were formed for the first time. Partial demethylation of the derivatives with sulphuric acid occurred in highly selective fashion at the methoxyl ortho to the halogen, without regard to the relative position of the carbonyl group which is known to be highly directive for demethylations in the absence of halogen. The phenolic products of the demethylations are needed for the synthesis of sterically hindered diphenyl ethers. They showed a type of intermolecular hydrogen bonding in the crystal which was markedly subject to steric interference by the halogen atoms.

Our work with the chlorinated derivatives of metameconine (X) was first undertaken many years ago as a check upon the results we had obtained in the corresponding bromine series (1, 2, 3); these results had differed sharply from those reported by others (4). The need for a check was emphasized when our more recent work (5) led to somewhat surprising conclusions as to the direction of bromination, nitration, and selective demethylation in the metameconines. This paper reports the independent characterization of the chlorine series of compounds, shows that the chlorine series is an almost exact parallel to the bromine series, and therefore thoroughly verifies our previous results.

Two more reasons for proceeding with the chlorine series appeared later in our work. (*a*) A subject of continuing interest to us is the study of steric hindrance in diphenyl ethers (3, 6); obviously the ortho-chloro- and ortho-bromo-phenols obtained in this work will allow the preparation of corresponding diphenyl ethers with blocking groups of different sizes. (*b*) We hope to study the effect of crowding upon the quadrupole resonance frequency of the halogens; measurement of these frequencies is, of course, easier for chlorine compounds than for bromine or iodine compounds.

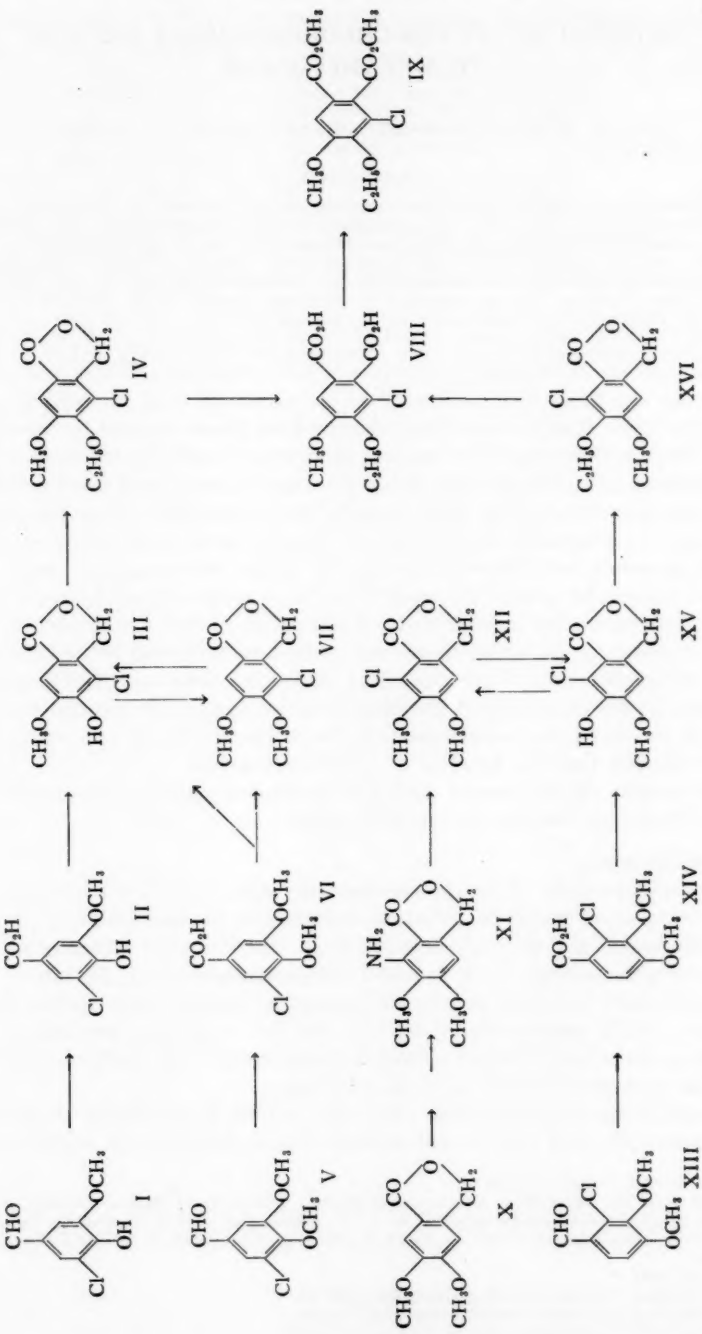
The main novelty of the present work lies in the description of an interesting steric hindrance of hydrogen bonding in the solid phase.

Synthesis and Structure

The most easily available of the chlorine derivatives was 7-chlorometameconine[5] (XII), made from metameconine (X) by nitration, reduction to the amine (XI) (1, 2, 4), diazotization of the amine, and the Sandmeyer reaction. No doubt the structure was obvious, since 7-bromometameconine (1, 2, 4) and 7-iodometameconine (1, 3), formed in corresponding ways, have had their structures thoroughly verified. Nevertheless the present work offered a quite independent proof that the new substance was indeed 7-chlorometameconine, its indirect formation from 2-chloroveratric acid (XIV), as shown in the chart, leaving no room for doubt as to its structure.

The isomeric 4-chlorometameconine (VII) was formed by the action of formaldehyde upon 5-chloroveratric acid (VI) in acid solution. The structure of the resulting phthalide

[1]*Manuscript received January 12, 1961.*
Contribution from the Department of Chemistry, Queen's University, Kingston, Ontario, Canada. The material in this paper is taken from the Report of the C.I.L. Fellow, Part I (R.Y.M., Queen's University, May, 1944) and from a forthcoming thesis for the degree of Doctor of Philosophy at Queen's University, by Mrs. Marjorie Allen.
[2]*Died April 8, 1960.*
[3]*Holder of Canadian Industries Limited Fellowship 1958–60.*
[4]*Holder of Canadian Industries Limited Fellowship 1942–43.*
[5]*A different numbering scheme was used in some earlier papers.*



was not immediately obvious, since the phthalide ring might have closed on either of the positions ortho to the carbonyl group in VI. That it did actually close as shown in structure VII followed from the fact that the phthalide could be converted to the phthalic ester IX which (as shown on the chart) could also be formed from metameconine (X) or from 2-chloroveratric acid (XIV).

Interesting phenolic compounds were obtained by selective demethylation of the chlorinated metameconines with sulphuric acid. Preparative yields were obtained of the easily purified products, much desired for future synthetic work. From 4-chlorometameconine (VII) there was obtained 4-chloro-5-hydroxy-6-methoxyphthalide (III), and from 7-chlorometameconine (XII) the isomeric 7-chloro-6-hydroxy-5-methoxyphthalide (XV). Just as in the bromine series, demethylation of both VII and XII occurred overwhelmingly at the methoxyl group ortho to the halogen, and without regard to the position of the carbonyl group, even though with veratraldehyde (7), veratric acid (7), and metameconine (5), highly selective demethylation occurred at the methoxyl meta to the carbonyl.

The methods used to show the structures of the phenolic compounds will be obvious from the transformations shown on the chart, the properties given in the experimental part, and the description of the corresponding bromine compounds (5).

Formaldehyde and 2-chloroveratric acid in acid solution gave none of the expected 7-chlorometameconine (XII); instead, very small amounts of 7-chloro-6-hydroxy-5-methoxyphthalide (XV) were obtained, along with large amounts of tars; the product was exceedingly difficult to purify. This miserable reaction was of central importance in our investigation, since it formed an exact parallel with the corresponding reaction of formaldehyde and 2-bromoveratric acid (1, 6), one of the main points in which our previous work disagreed with that of Rây and Robinson (4). Formaldehyde and 5-chloroveratric acid gave a mixture of the expected 4-chlorometameconine (VII) and the partially demethylated product, 4-chloro-5-hydroxy-6-methoxyphthalide (III). This too was an exact parallel with our experience with the corresponding 5-bromoveratric acid (2, 5). Even the infrared spectra of corresponding bromine and chlorine compounds are very similar throughout the two series, a further confirmation of the work in both. In fact the parallelism of the series broke down in only one respect: reductive dehalogenation, easy and convenient in the bromine series (5), in our hands failed completely in the chlorine series.

The correct orientation of the compounds of the metameconine series (1, 2, 3, 5, 6) has rested upon the correct orientation of the derivatives of vanillin used as reference compounds. It seems proper to point out how heavily we have relied upon the careful work of Dr. Raiford[6] and his students; of some 25 derivatives of vanillin needed in our structure proofs, only two (noted in the experimental part) have differed significantly from the descriptions given by these workers. One of the necessary intermediates, 5-chlorovanillic acid (II), has been made for the first time by Pearl's method (10), as shown in the experimental part.

Physical Properties

Jones, Angell, Ito, and Smith (12) reported that unsaturated lactones in solution sometimes show double peaks in the region of the carbonyl stretching frequency, though the two phthalides they investigated both showed single peaks. When their paper appeared

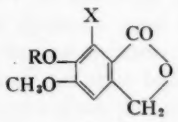
[6]*We especially wish to acknowledge the kindness of Professor Raiford in sending us an authentic sample of 2-chloroveratric acid nearly a year before its description was published (8), at a time when our work was delayed by the erroneous description of the acid then available (9), and not long before Professor Raiford's death (11).*

we had already observed, among the spectra of about 20 *solid* phthalides, a number of double peaks as well as some exceptionally large displacements of the carbonyl stretching frequency. A suggestion by Dr. R. N. Jones then enabled us to gain some understanding of the phenomena we had observed. Repetition of the spectra of the phthalides, but using solutions in tetrahydrofuran rather than solid samples in potassium bromide, immediately eliminated both the doubling of the peaks and the large displacements. Both factors were therefore due to intermolecular effects in the crystals; they were not due to impurities, incorrectly determined structures, or to unusual situations within the molecules.

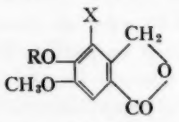
If one is interested in infrared spectra only for the determination of molecular structures, then the spectra of the crystals might simply be dismissed as inferior to the spectra of solutions. But the crystal effects are interesting in themselves, and had a special urgency for us, since quadrupole resonances *must* be observed in the solid state. We were able to make some progress in understanding the crystal effects because of the very large number of closely related phthalides at our disposal.

Spectra of Phthalides in Tetrahydrofuran

No double peaks were observed in the carbonyl stretching region, even in the acetylated derivatives (XVII A*d*, XVII C*d*, and XVIII C*d*) which have two different carbonyl groups. The peaks were all at positions normal for phthalides (13). In view of the results



XVII



XVIII

Substituent code

A: X = H	*a*: R = H
B: X = Cl	*b*: R = Me
C: X = Br	*c*: R = Et
D: X = I	*d*: R = Ac

Examples: XVII A*b* is structure X; XVII B*c* is XVI.

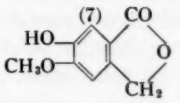
with the solid phthalides reported below, it was especially noteworthy that the phthalides bearing free phenolic groups had their peaks in the normal position, in agreement with previous work on related compounds (13, 14). Twelve halogenated phthalides (XVII and XVIII, with all possible combinations of B, C, *a*, *b*, and *c*) had their maxima in the region 1769–1773 cm^{-1}. Three unhalogenated phthalides (XVII A*a*, XVII A*b*, and XVIII A*a*) absorbed in the region 1766–1769 cm^{-1}. The available acetylated phthalides (XVII A*d*, XVII C*d*, and XVIII C*d*) showed absorption at the noticeably higher values of 1775–1777 cm^{-1}.

Spectra of Solid Phthalides in Potassium Bromide

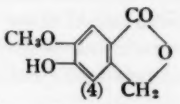
We discuss only effects which are both large and reproducible, since the peaks were, of course, broader and had less sharply defined maxima than those obtained with solutions, and because of the well-known smaller spectral variations possible with this technique. Two remarkable phenomena were noticed.

(*a*) *Steric interference with intermolecular hydrogen bonding.*—The reality of this effect was evident because the effect was reproducible, extremely large, and beautifully correlated with structure. Two phthalides (XVII A*a* and XVIII A*a*) (5) both showed absorption at the abnormally low frequency of 1715 cm^{-1}. The displacement of the band was

due to an intermolecular effect, since it vanished in solution; even phthalides having hydroxyl ortho to the carbonyl show but little intramolecular association (13, 14). Clearly too the displacement involved the hydroxylic hydrogen since methylation of the free hydroxylic group returned the maximum absorption (of solid XVII A*b*) to 1755 cm^{-1};



XVII A*a*



XVIII A*a*

acetylation of the free phenolic group brought the peak (in solid XVII A*d*) to 1753 cm^{-1}. Both values represent complete elimination of the effect. Direct hydrogen bonding from the phenolic group to the carbonyl group of another molecule would in any case be suspected from the great effect on the carbonyl stretching frequency, and confirmation of this was afforded by a most interesting steric effect. Substitution of either chlorine or bromine at position 7 in XVII A*a* also abolished the displacement effect, even though the hydroxyl group was still present, since solid XVII B*a* absorbed at 1760 cm^{-1}, and solid XVII C*a* at 1768 cm^{-1}. In view of our other results, by far the main effect of halogen at position 7 must have been due to steric blocking of the carbonyl group. Halogen at position 4 was very much less effective in interfering with the hydrogen bonding, solid XVIII B*a* absorbing at 1726 cm^{-1}, and solid XVIII C*a* at 1730 cm^{-1}, and this comparatively small increase from the values for unsubstituted XVIII A*a* may have been due to a combination of effects: a partial steric interference with the phenolic group, a general increase in lattice dimensions, and a small direct effect of halogen on the carbonyl group (see solution values, and reference 13). We have not yet been able to form phthalides having halogen ortho to carbonyl and meta to hydroxyl, so that these three effects cannot yet be separated from each other. Nevertheless, the displacement of the carbonyl peak in the solid phenolic compounds is so clear cut that it might have been used in the structure proofs to fix the relative positions of halogen and carbonyl. Eight solid phthalides in which intermolecular hydrogen bonding was absent showed a single peak in the carbonyl stretching region in the range 1753–1768 cm^{-1}. The rather wide range may be correlated with the lattice effects discussed in the next section.

(*b*) *Double carbonyl peaks with solid phthalides.*—Seven solid phthalides showed very distinct double carbonyl peaks; of the seven all were halogenated, none was phenolic, and none was acetylated. The lower peaks were all in the region 1748–1755 cm^{-1}, but the upper peaks were more variable in position. Four phthalides halogenated at position 4 (XVIII B*b*, B*c*, C*b*, and C*c*) had the upper peak at 1776–1782 cm^{-1}, and three phthalides halogenated at position 7 (XVII B*b*, C*b*, and D*b*) had the upper peak in the noticeably lower range of 1764–1767 cm^{-1}. The peaks varied considerably in relative amplitudes, without any obvious correlation with structure.

The double peaks were certainly due to effects of crystal structure, since only a single peak appeared in the spectra of solutions. For at least one phthalide, the double peak was associated with dimorphism. Fresh preparations of XVIII C*c* (5) consisted of feathery needles and showed peaks at 1753 and 1781 cm^{-1}. Older preparations consisted of heavy prisms and showed only a single peak at 1758 cm^{-1}. We have been unable to obtain a single peak in the spectrum of the less stable form. On the other hand the phthalide XVIII C*d* (5) showed only a single peak at room temperature, though it showed dimorphism at higher temperatures, the long needles melting at 140°, then resolidifying to

small prisms which melted again at 165°. Although these have been our only direct observations of dimorphism, the phenomenon cannot be ruled out as the explanation of all the double peaks observed with our phthalides.

Double Carbonyl Peaks in the Phthalic Esters

The dimethyl ester of 3-chloro-4-ethoxy-5-methoxyphthalic acid showed double peaks both in solution (at 1730 and 1744 cm^{-1}) and in the solid state (at 1720 and 1735 cm^{-1}); the corresponding bromine compound (5) was very similar in its spectra. The corresponding phthalic acids showed double peaks in the solid state, and single, very wide peaks in solution.

EXPERIMENTAL

All identities claimed in this paper were checked by comparison of the infrared spectra. The spectra were obtained using potassium bromide disks containing 0.8% by weight of sample, and tetrahydrofuran solutions containing up to 6% depending on the solubility of the sample. The spectrometer (a Perkin–Elmer Model 21, using a sodium chloride prism) was repeatedly calibrated by the use of the polystyrene bands at 1802 cm^{-1} and 1603 cm^{-1}. Most melting points were determined in an instrument of high precision (15) and the rest were performed on a Koffler Micro Hot Stage and lowered 3–5° to agree with precision capillary melting points within less than 1° up to 250°.

5-Chlorovanillic Acid (II)

The oxidation of 5-chlorovanillin (I) (m.p. 162.6–163.2°) (16) was accomplished following the method described by Pearl (10) for the preparation of vanillic acid. The product melted at 252–254°. The recorded (17) melting point is 244–245°. Found: C, 47.58; H, 3.39; Cl, 17.25%. Calc. for $C_8H_7ClO_4$: C, 47.42; H, 3.48; Cl, 17.52%.

5-Chloroveratraldehyde (V)

The method described for veratraldehyde (18) was adapted to the methylation of 5-chlorovanillin. The product melted at 55–55.6°; the recorded (11) melting point is 48–49°. Found: Cl, 18.2%. Calc. for $C_9H_9ClO_3$: Cl, 17.69%.

5-Chloroveratric Acid (VI)

5-Chloroveratraldehyde (17.0 g) and sodium bicarbonate (3.0 g) in water (100 ml) were heated to 60°. With vigorous stirring a solution, also heated to 60°, of potassium permanganate (20.2 g) in water (200 ml) was slowly added over 30 minutes whilst the temperature was maintained at 60–80°. The reaction was allowed to proceed for another 30 minutes, and then the reaction mixture was cooled, saturated with sulphur dioxide, and strongly acidified with hydrochloric acid (20 ml, conc.). The white precipitate was recovered by filtration, washed with a small volume of cold water and crystallized from ethyl alcohol to yield the product (15.6 g) with a melting point of 189.0–189.6°. Two more crystallizations from ethyl alcohol raised the melting point to 189.8–190.4°. The recorded (11) melting point is 188–189°. Found: C, 50.14; H, 4.00; Cl, 16.38%. Calc. for $C_9H_9ClO_4$: C, 49.88; H, 4.19; Cl, 16.38%.

2-Chloroveratric Acid (XIV)

This acid was obtained from 2-chlorovanillin by methylation according to the procedure recorded for veratraldehyde (18) followed by oxidation by the method described above for the isomeric 5-chloroveratric acid. The product purified by crystallization from aqueous ethyl alcohol had a melting point of 202°–203°. A mixed melting point determination with an authentic sample supplied by Professor Raiford gave a melting point of 202.5–204.5°.

4-Chloro-5-hydroxy-6-methoxyphthalide (III)

(*a*) 5-Chlorovanillic acid (II) (2.0 g, m.p. 247–249°), glacial acetic acid (80 ml), hydrochloric acid (40 ml, conc.), and formaldehyde (20 ml, 36–38%) were mixed and heated at 80–85° for 2 days. The cooled reaction mixture was poured onto ice (200 g) and after 2 hours the resulting precipitate was recovered by filtration, washed with a little cold water, and dried. The product (1.18 g) had a melting point of 204–206° which was raised to 236.4–237.2° by three crystallizations from ethyl alcohol. Found: C, 50.55, 50.78; H, 3.55, 3.46; Cl, 16.21, 16.55; —OCH_3, 15.45, 15.37 and on the same sample by a different analyst 13.64%. Calc. for $C_9H_7ClO_4$: C, 50.37; H, 3.29; Cl, 16.54; —OCH_3, 14.46%.

(*b*) 5-Chloroveratric acid (VI) (10 g, m.p. 186–187°), glacial acetic acid (400 ml), hydrochloric acid (200 ml, conc.), and formaldehyde solution (100 ml, 36–38%) were combined and heated at 95–100° for 2 days. The white precipitate obtained after pouring the reaction mixture onto ice was extracted with sodium bicarbonate solution. The insoluble fraction yielded after one crystallization from ethyl alcohol 4-chlorometameconine (VII) (1.66 g) with a melting point of 168–171°. (For further details see below.) The sodium bicarbonate extract was acidified and the resulting precipitate recovered and crystallized from ethyl alcohol. The product (2.77 g) had a poor melting point of 168–220°, but a second crystallization raised the melting point to 234–235°. A mixed melting point with the product from method (*a*) showed no depression.

(*c*) 4-Chlorometameconine (VII) (0.13 g, m.p. 171.2–171.8°) was heated in sulphuric acid (1 ml, conc.) at 60° for 2 days. The solution was poured onto ice and the precipitate recovered by filtration and washed with water. The product after two crystallizations from ethyl alcohol melted at 234–235° and gave no depression in melting point when mixed with the product from method (*a*).

The infrared spectra of the three samples of 4-chloro-5-hydroxy-6-methoxyphthalide produced by these methods, are identical throughout the range 4000–600 cm^{-1}.

4-Chlorometameconine (VII)

(*a*) This method of preparation is the same as method (*b*) described for 4-chloro-5-hydroxy-6-methoxyphthalide. The product (1.66 g) was crystallized twice from ethyl alcohol to give the analytical sample with a melting point of 171.2–171.8°. Found: C, 52.77, 52.71; H, 4.11, 4.25; Cl, 15.62, 15.65%. Calc. for $C_{10}H_9ClO_4$: C, 52.50; H, 3.97; Cl, 15.52%.

(*b*) 4-Chloro-5-hydroxy-6-methoxyphthalide (III) (0.2 g, m.p. 234–235°) from method (*a*) above was added to an ether solution of diazomethane (20 ml, 2%) and the mixture was left to stand overnight. The solution was evaporated and the easily solidified residue crystallized from ethyl alcohol to give fluffy needles (0.13 g) which melted at 171.2–171.8°. A mixed melting point with the product from (*a*) gave no depression, and the infrared spectra of the two products were identical.

4-Chloro-5-ethoxy-6-methoxyphthalide (IV)

4-Chloro-5-hydroxy-6-methoxyphthalide (III) (1.63 g, m.p. 234–235°), from method (*a*) above, ethyl iodide (30 ml), and dry acetone (75 ml) were refluxed and stirred in dry apparatus protected from moisture by drying tubes. Drierite (1.0 g) was added and the mixture stirred for 30 minutes. Freshly prepared silver oxide (5.0 g) was added in portions (1.0 g) at intervals over 7 hours, the reaction was allowed to proceed for a further 2 hours, and then the reaction mixture was cooled and filtered to remove the silver compounds. The filtrate was evaporated to dryness and the solid residue crystallized from ethyl alcohol to give white crystals (1.48 g) which melted at 138–139°. Three more

crystallizations raised the melting point to 141.4–141.8°. Found: C, 54.72, 54.67; H, 4.70, 4.45; Cl, 14.30%. Calc. for $C_{11}H_{11}ClO_4$: C, 54.44; H, 4.57; Cl, 14.63%.

7-Chlorometameconine (XII)

(*a*) 7-Aminometameconine (XI) (2) (15 g crude) was dissolved in hydrochloric acid (45 ml, conc.) and water (300 ml). The solution was mechanically stirred and cooled in an ice and salt bath to −5°. Sodium nitrite (7.5 g) in water (60 ml) was added from a dropping funnel over 20 minutes, and then the mixture was stirred and cooled for 3.5 hours longer. The excess nitrous acid was destroyed with urea and the reaction mixture was slowly poured, with stirring, into a well-cooled solution of cuprous chloride (from 19 g $CuSO_4.5H_2O$) in hydrochloric acid (21 ml, conc.) and water (8 ml). When the frothing had subsided, the reaction mixture was gently heated on a boiling-water bath. The precipitate which settled out of the cooled solution, was recovered by filtration, washed with water, and crystallized once from dilute acetic acid (3:2) and three times from *n*-butanol to yield white needles (5.0 g) with a melting point of 193–193.5°. Two more crystallizations of corresponding material from another preparation gave the analytical sample with a melting point of 195.8–196.6°. Found: C, 52.48, 52.32; H, 3.97, 3.96; Cl, 15.22, 15.37%. Calc. for $C_{10}H_9ClO_4$: C, 52.50; H, 3.97; Cl, 15.52%.

(*b*) 5-Methoxy-6-hydroxy-7-chlorophthalide (XV) (0.5 g, m.p. 234–235°) from method (*a*) below, when methylated with diazomethane yielded white needles (0.42 g), with a melting point of 194.2–195.2° after one crystallization from ethyl alcohol. The infrared spectra of these two samples of 7-bromometameconine are identical, and a mixed melting showed no depression. The material made by method (*b*) below was methylated with methyl sulphate and gave 7-chlorometameconine proved by mixed melting point determination and infrared spectra.

5-Methoxy-6-hydroxy-7-chlorophthalide (XV)

(*a*) 7-Chlorometameconine (XII) (2.0 g, m.p. 193–194°) was heated with sulphuric acid (10 ml, conc.) for 3.5 days at 60°. The cooled viscous solution was poured onto ice and the resulting precipitate recovered by filtration, washed with water, and dried. The solid (1.87 g) was extracted with sodium bicarbonate solution and the soluble fraction recovered by acidifying the extracts. This procedure yielded a solid (1.47 g) with a melting point of 230–231°, which was raised to 235–235.8° by three crystallizations from ethyl alcohol. Found: C, 50.66, 50.48; H, 3.42, 3.28; Cl, 16.23, 16.53; —OCH_3, 14.44%. Calc. for $C_9H_7ClO_4$: C, 50.37; H, 3.29; Cl, 16.54; —OCH_3, 14.46%. A mixed melting point with the isomer 4-chloro-5-hydroxy-6-methoxyphthalide (III) showed a large depression.

(*b*) 2-Chloroveratric acid (XIV) (5.0 g), formaldehyde solution (9.0 ml, 36–38%), hydrochloric acid (31 ml, conc.), and glacial acetic acid (45 ml) were heated at 80–90° for 76 hours and at 100° for 7 hours longer. The combined solutions from three similar experiments were cooled and decanted from a small amount of tar, diluted with water (85 ml), and filtered to remove more tar. A second dilution with water (50 ml) precipitated unchanged 2-chloroveratric acid which after successive crystallizations from ethyl alcohol, aqueous ethyl alcohol, *n*-butyl alcohol, and aqueous acetic acid melted at 200–201.5°. The filtrate was again diluted with water (500 ml) and the solution evaporated to less than 150 ml under a slight vacuum, neutralized with sodium bicarbonate, made very slightly acid with hydrochloric acid, and the resulting precipitate collected after 24 hours. The precipitate (1.95 g) was washed with hot and cold water, and crystallized three times from *n*-butanol to give a product with a melting point of 231–232.5°. Found: C, 51.98; H, 3.84; Cl, 16.85, 16.50%. Calc. for $C_9H_7ClO_4$: C, 50.37; H, 3.29; Cl, 16.54%.

Better material, m.p. 234.2–234.8°, was obtained by a further recrystallization from ethyl alcohol. The infrared spectra given by these two products from methods (*a*) and (*b*) were identical.

5-Methoxy-6-ethoxy-7-chlorophthalide (XVI)

5-Methoxy-6-hydroxy-7-chlorophthalide (XV) (2.0 g, m.p. 234–235°) was ethylated by the method described above for 4-chloro-5-hydroxy-6-methoxyphthalide. The product (1.59 g) after one crystallization from ethyl alcohol melted at 174–175°. Three more crystallizations raised the melting point to 177.2–177.6°. Found: C, 54.43, 54.52; H, 4.70, 4.56; Cl, 14.63%. Calc. for $C_{11}H_{11}ClO_4$: C, 54.44; H, 4.57; Cl, 14.63%.

3-Chloro-4-ethoxy-5-methoxyphthalic Acid (VIII)

(*a*) Potassium permanganate (2.5 g) was added over a period of 15 minutes to a well-stirred solution of 4-chloro-5-ethoxy-6-methoxyphthalide (IV) (1.0 g, m.p. 140.6–141.2°) in potassium hydroxide solution (110 ml, 5%) heated on a steam bath. The reaction was allowed to proceed another 45 minutes, and then cooled. Sulphur dioxide was passed until a clear solution was obtained. The solution was evaporated to half volume and after standing at room temperature, crystals with a poor melting point settled out of the solution. The product crystallized from water containing a few drops of hydrochloric acid in two modifications which were separated by hand: (A) long needles (0.2 g) with a melting point of 200–201° and a second melting point due to the anhydride of 133.5–134°; (B) small prisms (0.02 g) with a melting point of 203–207° and a second melting point of 133.5–134°. The infrared spectra in potassium bromide disks of these two modifications showed slight differences in the intensity of the peaks in the region 1500–1450 cm^{-1}, and differences in the positions of the peaks in the region 1000–850 cm^{-1}, which are probably due to variations in the crystalline structure. The two forms were methylated separately (see below) to give identical products as determined by infrared spectra and mixed melting points.

(*b*) 5-Methoxy-6-ethoxy-7-chlorophthalide (XVI) (1.0 g, m.p. 174–175°) was oxidized as above, and the product after one crystallization from water melted at 192–194° and the second melting point was 133.5–134°. This product appeared to be homogeneous, and the infrared spectrum was identical with that given by the modification B above.

Dimethyl 3-Chloro-4-ethoxy-5-methoxyphthalate (IX)

(*a*) 3-Chloro-4-ethoxy-5-methoxyphthalic acid (0.2 g, crystals A) were added to diazomethane solution (20 ml, 2% in ether) and left to stand overnight. The colorless solution was evaporated to dryness and the easily solidified residue was crystallized from petroleum ether (b.p. 30–60°) to give heavy needles which melted at 68.8–70.4°.

The product from crystals B melted at 70.4–70.6°, and a mixed melting point was 68.8–70.4°.

(*b*) 3-Chloro-4-ethoxy-5-methoxyphthalic acid (0.44 g from (*b*) above) yielded white needles (0.38 g) with a melting point of 70.4–71.4°, which did not depress the melting point of the above products. Found: C, 51.70, 51.66; H, 5.12, 4.86; Cl, 12.29, 12.42%. Calc. for $C_{13}H_{15}ClO_6$: C, 51.56; H, 4.99; Cl, 11.72%.

The infrared spectra given by the three samples of dimethyl 3-chloro-4-ethoxy-5-methoxyphthalate were identical.

Attempted Dechlorinations

5-Chlorovanillic acid (II) was used as a model compound in experiments designed to find the conditions for removal of the chlorine atom. The methods tried were: (*a*) sodium

amalgam in aqueous medium, (*b*) zinc dust and potassium hydroxide, (*c*) preformed chromous chloride, (*d*) chromous chloride prepared *in situ*. All experiments failed, the only compound isolated being unchanged 5-chlorovanillic acid. The last two series of experiments were repeated with 4-chloro-5-hydroxy-6-methoxyphthalide (III) and once again there was no evidence that chlorine had been successfully removed.

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THE CARBOHYDRATE–PROTEIN LINKAGE IN GLYCOPROTEINS

PART I. THE SYNTHESES OF SOME MODEL SUBSTITUTED AMIDES AND AN L-SERYL-D-GLUCOSAMINIDE[1]

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ABSTRACT

The syntheses of *N*-L-seryl-D-glucosamine, *N*-glycyl-D-glucosamine, *N*-L-glutamyl-D-glucosamine, and 1-*O*-β-L-seryl-*N*-acetyl-D-glucosaminide are described. These D-glucosamine derivatives may result from the fragmentation of some glycoproteins. The chemical properties of these compounds indicate that *N*-peptides are relatively stable in acid solution but unstable in neutral and alkaline solution. The seryl glucosaminide is relatively stable in alkaline solution but is hydrolyzed by acid.

INTRODUCTION

The mode of the linkage of sugars to amino acids in glycoproteins and mucoproteins which contain small amounts of carbohydrate, is obscure. Examples of this group are collagen and reticulin. Reticulin (1) is found in fairly large amounts in some internal organs (e.g. kidney and lung), and contains 3–4% of neutral sugars and 0.2% of hexosamine. Collagen (2) contains 0.3–0.7% of glucose and galactose. Several collagens which have been investigated (3) have been shown to contain D-glucosamine. Histochemical work has demonstrated that in some diseases, such as rheumatoid arthritis, there appear to be increases in the carbohydrate content of the glycoprotein of the subcutaneous tissue, which is rich in collagen. It was therefore of interest to investigate the mode of linkage of carbohydrate to amino acid in glycoproteins and mucoproteins. Bovine subcutaneous tissue, which is rich in collagen and elastic fibers, when degraded by proteolytic enzymes yielded a complex mixture of peptides to which were attached sugar residues. It was clear that this subcutaneous tissue did not contain any appreciable quantities of high molecular weight polysaccharides (4). This suggested that carbohydrate residues of low molecular weight were covalently linked to the amino acid residues of the proteins. It was therefore decided to attempt the syntheses of carbohydrate–amino acid derivatives which might result from the degradation of glycoproteins. A survey of the literature indicates that little was known about the type of linkage in such a derivative, but that linkages through glycine, aspartic acid, serine, threonine, and amides have been implicated (5, 6, 7). Many glycoproteins contain D-glucosamine and the possible biological role of *N*-peptides of this amino sugar have been investigated. The syntheses of *N*-glycyl-D-glucosamine (8) and of *N*-L-alanyl-D-glucosamine (9) have been reported in which they were isolated either as the hydrochloride or as some alternative derivative, with the amino group protected. *N*-(L-Alanyl-L-alanyl)-D-glucosamine has been found to be stable (10), as have several other similar dipeptides, the syntheses of which have been reported. A peptide composed of D-glucosamine and L-glutamic acid has been synthesized (11) but this was reported to be unstable even when the amino group was protected. It was of particular interest to examine these *N*-peptides, rather than their more stable substituted derivatives to determine whether they might be expected to survive during the hydrolysis

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of glycoproteins. Accordingly, we have attempted the syntheses of several amino acids amide linked to D-glucosamine, in order to examine their properties. The *N*-peptide of L-serine was chosen as it had been reported by Masamune (12) to be present in gastric mucin together with glucosamine. Glycine has been reported by Gross (13) to constitute about 30–35% of all collagens investigated, and may therefore be linked to sugar residues. The *N*-peptide of L-glutamic acid was prepared in order to investigate whether the presence of a free carboxylic acid end group might confer stability to the derivative. L-Serine contains a primary hydroxyl group, and is therefore capable of being united glycosidically with a sugar residue: an L-seryl-*N*-acetyl-D-glucosaminide has been synthesized and its properties examined.

N-L-Seryl-D-glucosamine

N-(*N*-Carbobenzoxy-L-alanyl)-1,3,4,6-tetra-*O*-acetyl-β-D-glucosamine hydrochloride and the corresponding *N*-glycyl compound were synthesized by Bergmann and Zervas (8) by treating 1,3,4,6-tetra-*O*-acetyl-β-D-glucosamine with *N*-carbobenzoxy-L-alanyl chloride or with *N*-carbobenzoxyglycyl chloride. L-Serine, however, contains a primary hydroxyl group which must be protected before a reaction of this type can be used. Accordingly we turned our attention to the possibility of condensing a suitably substituted D-glucosamine derivative with *N*-carbobenzoxy-L-serine by the carbodiimide method (14). Unfortunately the L-serine derivative was not soluble in methylene chloride which is the solvent usually employed in this type of reaction. To overcome this difficulty the primary hydroxyl group of the L-serine derivative was protected by a tetrahydropyranyl group which led to the formation of two separable optical isomers which were soluble in methylene chloride. Racemization does not occur in the formation of the tetrahydropyranyl derivatives (15). Each isomer was then condensed with the suitably substituted 1-*O*-α-acetyl- and 1-*O*-β-acetyl-D-glucosamine derivatives, resulting in the formation of four isomers. The yields were poor when dicyclohexyl carbodiimide was used because of the difficulty in separating dicyclohexyl urea from the products. When diisopropyl carbodiimide was used as a condensation agent, better yields were obtained since the product was more readily separable from the diisopropyl urea by-product. When these isomers were warmed in dilute acetic acid solution, dihydropyran was eliminated and *N*-(*N*-carbobenzoxy-L-seryl)-1,3,4,6-tetra-*O*-acetyl-α- (and β-) D-glucosamine were produced, respectively. When the carbobenzoxy grouping was removed by reduction the resulting *N*-L-seryl-1,3,4,6-tetra-*O*-acetyl-α- (and β-) D-glucosamine decomposed to a brown tar within a few minutes. Presumably this was due to the basic character of the amino group of the amino acid moiety. Despite this instability it was possible to prepare the fully acetylated derivative. However, it was found that in the presence of hydrochloric acid the deacetylated product yielded a stable hydrochloride on removal of the carbobenzoxy grouping by hydrogenolysis.

N-Glycyl-D-glucosamine

Bergmann (8) synthesized the hydrochloride of this material but the parent compound has not been reported. *N*-(*N*-Carbobenzoxyglycyl)-D-glucosamine was obtained by the deacetylation of *N*-(*N*-carbobenzoxyglycyl)-1,3,4,6-tetra-*O*-acetyl-β-D-glucosamine. This compound was prepared by Bodanszky and Du Vigneaud's method (16) for condensing amino groups and carboxylic acid groups to form peptide linkages. The condensation is effected between the hydrogen halide of an amine and the *p*-nitrophenyl ester of a carboxylic acid. In this case the amine salt used was 1,3,4,6-tetra-*O*-acetyl-β-D-glucosamine hydrobromide, and the ester was glycyl *p*-nitrophenyl ester. The fully

substituted product was then deacetylated and reduced in the presence of hydrochloric acid to give *N*-(glycyl hydrochloride)-D-glucosamine. Hydrogenolysis in neutral solution caused both the acetylated and the deacetylated derivative to decompose rapidly.

N-L-Glutamyl-D-glucosamine

In the synthesis of this compound the method of Bodanszky and Du Vigneaud (16) was again used in an analogous manner to that used in the preparation of the *N*-glycyl-D-glucosamine. The amino acid was converted into *N*-carbobenzoxy-α-*p*-nitrophenyl ester-γ-L-glutamine by condensation of *N*-carbobenzoxy-L-glutamic acid with *p*-nitrophenol, using diisopropyl carbodiimide as a dehydrating agent, to form the diester. The diester was then treated with the calculated amount of methanolic ammonia solution to form the monoester–monoamide. It is reasoned from steric considerations that the replacement is effected in the less hindered γ-position. When the derivative had been formed and the terminal amide group removed, attempts to synthesize the free amine by hydrogenolysis of the carbobenzoxy group yielded very unstable products which could not be isolated. This was also true of the *N*-peptide after deacetylation. The hydrogen reduction was also carried out in the presence of hydrochloric acid, and in this case the stable *N*-peptide hydrochloride was formed.

1-O-β-L-Seryl-N-acetyl-D-glucosaminide

The glucosaminide was formed using a modified Koenigs–Knorr (17) type synthesis. 1-Chloro-1-deoxy-*N*-acetyl-3,4,6-tri-*O*-acetyl-D-glucosamine was prepared by the action of titanium tetrachloride on fully acetylated D-glucosamine in the manner described by Inouye and his co-workers (18). The acetohalogen sugar was allowed to react with *N*-carbobenzoxy-L-seryl methyl ester, using anhydrous alcohol-free chloroform as a solvent, and employing the Koenigs–Knorr conditions.

The initial yields of the product were low owing to steric hindrance, but when the reaction was allowed to proceed for 6 days with vigorous shaking, better yields were obtained. The product was not purified at this stage, but was de-*O*-acetylated with barium methylate. The deacetylated material was purified by chromatography, and was then subjected to hydrogenolysis, under neutral conditions, to remove the carbobenzoxy group. The over-all yield was small and the product at this stage, 1-*O*-β-(L-seryl methyl ester)-*N*-acetyl-D-glucosaminide, was stable and the corresponding hydrochloride was synthesized. The substituted D-glucosaminide was then saponified using sodium hydroxide solution to give the free acid, 1-*O*-β-L-seryl-*N*-acetyl-D-glucosaminide, which was found to be stable.

EXPERIMENTAL

All melting points reported are uncorrected. Solutions were concentrated by rotary evaporation under reduced pressure below 40°, unless otherwise stated.

Paper chromatography was performed by the descending method on Whatman No. 1 paper, using 1-butanol/ethanol/water (3:1:1 v/v) as the mobile phase. The following sprays were used to detect the compounds on the paper chromatograms: (A) 2% solution of *p*-anisidine hydrochloride in 1-butanol, (B) 1% solution of silver nitrate in acetone, followed by 2% ethanolic sodium hydroxide, and (C) 2% ninhydrin in 1-butanol. The rate of movement of the compounds on the chromatograms is given relative to that of D-glucosamine hydrochloride ($R_{GN.HCl}$), *N*-acetyl-D-glucosamine (R_{GNAc}), or pentaacetyl-D-glucosamine (R_{GNpAc}).

The infrared spectra of crystalline compounds were measured as 0.8% dispersions

in potassium bromide disks, and non-crystalline materials were examined as 6% solutions in chloroform, using a Perkin–Elmer Model 21 spectrophotometer.

Elemental analyses were carried out by the Schwarzkopf Microanalytical Laboratory, Woodside, New York.

1. N-L-Seryl-D-glucosamine

(a) Carbobenzoxychloride

The reagent was prepared by the method of Carter *et al.* (19). Benzyl alcohol (65 ml) was added to a solution of phosgene (69 g) in toluene (200 ml) at 0°. After 3 hours the excess benzyl alcohol and the toluene were removed by distillation under reduced pressure. The remaining solution (104 g) contained 76 g of carbobenzoxychloride.

(b) N-Carbobenzoxy-L-serine

The method of Moore *et al.* (14) was used in a slightly modified form. Between the additions of the reactants the alkaline solution was transferred to a tap funnel and vigorously shaken, then returned to the reaction vessel. The product (98% yield) had m.p. 116°.

(c) N-Carbobenzoxy-L-seryl Methyl Ester

N-Carbobenzoxy-L-serine (20 g) was dissolved in 2.5 *N* methanolic hydrogen chloride solution (200 ml). The solution was allowed to stand overnight and it was then deacidified with Duolite A4(OH^-) exchange resin. The solution was concentrated to a syrup (20 g; 94% yield) which was kept in a desiccator.

(d) N-Carbobenzoxy-O-tetrahydropyranyl-L-seryl Methyl Ester

The procedure of Iselin and Schwyzer (15) was followed. *N*-Carbobenzoxy-L-seryl methyl ester (12.6 g) was dissolved in purified 2,3-dihydro-4-pyran (6 g) and a solution of 2.2 *N* hydrogen chloride in dry ethyl acetate (0.7 ml) was added. The reaction mixture was allowed to stand for 3 hours at room temperature after which it was diluted with ether and then washed with dilute sodium bicarbonate solution followed by water. The solution was dried (anhyd. sodium sulphate), filtered, and then concentrated to a yellow oil (15 g; 88% yield).

(e) N-Carbobenzoxy-O-tetrahydropyranyl-L-serine

The methyl ester (*d*) was dissolved in methanol (50 ml) and saponified by the method of Iselin and Schwyzer (15). Addition of ether to the final product in ethyl acetate resulted in the separation of one isomer in a crystalline form (I_{e1}), in 14% yield, which had m.p. 129°.

The second isomer (I_{e2}) was obtained from the concentrated mother liquor which gave crystals after 2 days (30% yield) which had m.p. 150°.

The infrared spectra of the products showed absorption bands at 987 cm^{-1} and 1130 cm^{-1} (in chloroform solution). These bands are due to the tetrahydropyranyl group, and are not present in the spectrum of *N*-carbobenzoxy-L-serine. The phenyl band at 695 cm^{-1} was also present.

(f) 1,3,4,6-Tetra-O-acetyl-N-acetyl-β-D-glucosamine

D-Glucosamine hydrochloride (30 g) was dissolved in a solution of zinc chloride (36 g) in acetic anhydride (250 ml). The solution was kept at 70–80° for 10 minutes and was then poured into ice water (400 ml). Sodium bicarbonate was slowly added to neutralize the excess acid and on allowing the reaction mixture to stand overnight in a refrigerator, a white precipitate of the pentaacetate separated. The material was collected, washed with water, dried, and recrystallized from ethanol solution to give the product (30.8 g;

52% yield) which had m.p. 187° which rose to m.p. 190–192° after recrystallization. $[\alpha]_D^{25}$ +3° (*c*, 4.0 chloroform).

The infrared spectrum of the product showed the following absorption bands: carbonyl of *O*-acetyl (1740 cm^{-1}); amide I (1682 cm^{-1}); and amide II (1512 cm^{-1}). Important bands referred to later occurred at 1238, 1078, 1042, and 900 cm^{-1}.

The corresponding α-anomer was prepared in a similar manner, but the reaction was carried out at room temperature. Yield 56%, m.p. 139°, $[\alpha]_D^{22}$ +91° (*c*, 4.0 chloroform).

(g) 1,3,4,6-Tetra-O-acetyl-α- (and β-) D-*glucosamine Hydrobromide*

The technique of Inouye *et al.* (18) was used. 1,3,4,6-Tetra-*O*-acetyl-*N*-acetyl-α-D-glucosamine (10 g) was added to a stirred ice-cold solution of dry hydrogen bromide in acetic acid (30 ml) to give the product (4.5 g; 45% yield), which had $[\alpha]_D^{22}$ +129° (*c*, 4.0 water), chars at 205–220°.

The β-anomer was prepared in a similar manner from 1,3,4,6-tetra-*O*-acetyl-*N*-acetyl-β-D-glucosamine and the product (4.5 g; 45% yield) had $[\alpha]_D^{21}$ +12.1° (*c*, 4.0 water), chars above 200°.

(h) 1,3,4,6-Tetra-O-acetyl-α- (and β-) D-*glucosamine*

The hydrobromides (1 g) were respectively dissolved in a slurry of Amberlite IR45 ($CO_3^=$) exchange resin (4 g hydrobromide to 50 ml resin). The mixture was stirred at 5° for 1 hour and then filtered. The filtrates were extracted with chloroform (4×80 ml) and the combined extracts were dried (anhyd. sodium sulphate) and then concentrated to a small volume. On the addition of ether to the concentrates, the products separated as needle-like crystals, which were collected and recrystallized from ethanol. The α-anomer (65% yield) had m.p. 118° and $[\alpha]_D^{23}$ +145.5° (*c*, 4.0 chloroform). The β-anomer (65% yield) had m.p. 143° and $[\alpha]_D^{22}$ +27° (*c*, 3.6 chloroform).

*(i) N-(N-Carbobenzoxy-O-tetrahydropyranyl-*L-*seryl)-1,3,4,6-tetra-O-acetyl-α- (and β-)* D-*glucosamine*

N-Carbobenzoxy-*O*-tetrahydropyranyl-L-serine (isomer $I_{\epsilon 1}$) (2.0 g; 6.2 mmoles), 1,3,4,6-tetra-*O*-acetyl-β-D-glucosamine (2.3 g; 6.2 mmoles), and diisopropyl carbodiimide (0.8 g; 6.2 mmoles) were dissolved in methylene chloride (25 ml) and the solution was shaken mechanically for 20 hours at room temperature. One drop of dilute acetic acid was added, and the shaking was continued for a further 1 hour. The diisopropyl urea by-product was filtered off, and the filtrate was stored at −10° for 24 hours. The solution was refiltered, and the filtrate then washed with dilute hydrochloric acid, followed by 2 *N* sodium carbonate solution and finally with water. The solution was dried (anhyd. sodium sulphate) and the methylene chloride removed by distillation to leave a syrup. The syrup was taken up in the minimum amount of hot ethanol from which the α-anomer was obtained as crystals (40% yield) which had m.p. 201° and $[\alpha]_D^{23}$ +10° (*c*, 4.0 chloroform). Anal. Found: C, 55.8; H, 6.16; N, 4.3%. $C_{30}H_{49}O_{14}N_2$ requires: C, 55.2; H, 6.18; N, 4.3%.

The above synthesis was repeated using 1,3,4,6-tetra-*O*-acetyl-α-D-glucosamine to give the α-anomer as a syrup (35% yield) which could not be crystallized and which had $[\alpha]_D^{23}$ +91° (*c*, 4.0 chloroform).

The second isomer ($I_{\epsilon 2}$) of *N*-carbobenzoxy-*O*-tetrahydropyranyl-L-serine was treated in the same way as described above with 1,3,4,6-tetra-*O*-acetyl-β-D-glucosamine to yield a crystalline product (29% yield) which had m.p. 160° and $[\alpha]_D^{23}$ +2° (*c*, 5.0 chloroform). Anal. Found: C, 55.5; H, 6.18; N, 4.6%.

The infrared absorption spectra of the products showed absorption band at the following wave numbers: carbonyl of *O*-acetyl (1745 cm^{-1}), amide I (1682 cm^{-1}), and amide II

(1495 cm^{-1}). The spectra showed the following absorption bands in common with 1,3,4,6-tetra-*O*-acetyl-β-D-glucosamine: 1235 (s), 1078 (s), 1041 (s), and 900 cm^{-1} (s). Bands characteristic of *N*-carbobenzoxy-*O*-tetrahydropyranyl-L-serine were detected at 1130, 987, and 695 cm^{-1}. The spectra of the isomers were almost identical.

*(j) N-(N-Carbobenzoxy-*L*-seryl)-1,3,4,6-tetra-O-acetyl-α- (and β-)* D*-glucosamine*

The *O*-tetrahydropyranyl group was removed from the products (*i*) by the following method. *N*-(*N*-Carbobenzoxy-*O*-tetrahydropyranyl-L-seryl)-1,3,4,6-tetra-*O*-acetyl-β-D-glucosamine (600 mg) was boiled for 10 minutes in 50% aqueous acetic acid (10 ml) and the cooled solution was then diluted with water (50 ml). The solution was extracted with chloroform (4×20 ml), the combined extracts were washed with water, dried (anhyd. sodium sulphate), and concentrated to a small volume. The residue was dissolved in the minimum quantity of hot ethanol from which the product crystallized slowly in the form of long colorless needles (75% yield) which had m.p. 168° and $[\alpha]_D^{23}$ +25° (*c*, 4.0 chloroform). Anal. Found: C, 53.3; H, 5.73; N, 5.0%. $C_{25}H_{32}O_{13}N_2$ requires: C, 52.8; H, 5.67; N, 4.93%.

The α-anomer was similarly prepared from *N*-(*N*-carbobenzoxy-*O*-tetrahydropyranyl-L-seryl)-1,3,4,6-tetra-*O*-acetyl-α-D-glucosamine as a crystalline product (65% yield) which had m.p. 145° and $[\alpha]_D^{23}$ +102° (*c*, 3.7 chloroform).

The infrared absorption spectra of these products were the same as those of the precursors except that the bands at 1130 cm^{-1} and 987 cm^{-1} due to the tetrahydropyranyl group were absent. The isomerism due to the forms (I_{e1}) and (I_{e2}) disappeared, as the same product was obtained from both isomers.

*(k) N-(N-Carbobenzoxy-*L*-seryl)-α- (and β-)* D*-glucosamine*

N-(*N*-Carbobenzoxy-L-seryl)-1,3,4,6-tetra-*O*-acetyl-α-D-glucosamine (0.4 g) dissolved in anhydrous methanol (8 ml) was treated with 0.4 *N* barium methoxide in methanol (2 ml) at 5° for 20 hours. After testing for the presence of excess barium, a little water was added and the remaining alkali was precipitated by carbonation of the solution. The barium carbonate was removed by centrifugation, and the clear supernatant was concentrated to a small volume. The residue gave a crystalline product (24% yield) from ethanol solution which had m.p. 195° and $[\alpha]_D^{20}$ +75° (*c*, 4.0 chloroform).

The β-anomer was similarly prepared from *N*-(*N*-carbobenzoxy-L-seryl)-1,3,4,6-tetra-*O*-acetyl-β-D-glucosamine as a crystalline product (30% yield) which had m.p. 198° and $[\alpha]_D^{21}$ +8° (*c*, 4.0 chloroform).

The products had R_{GNAc} 2.10 on paper chromatograms and was free from D-glucosamine and L-serine.

The infrared spectra of the two products were very similar, and differed from the spectra of the precursors in that the carbonyl of *O*-acetyl at 1750 cm^{-1} had disappeared, and the hydroxyl band at 3460 cm^{-1} appeared very strongly. The amide linkage bands at 1682 cm^{-1} and 1495 cm^{-1} were still present, though the latter was slightly displaced.

*(l) N-(*L*-Seryl hydrochloride)-*D*-glucosamine*

The carbobenzoxy group was removed from (1*k*) by hydrogenolysis using palladized charcoal as a catalyst. The catalyst was prepared by adding palladous chloride (0.1 g) in a little water to animal charcoal (2 g) and the mixture was then heated to 130° to remove water. Hydrazine sulphate was added, followed by a little *N* sodium hydroxide and the mixture was then stored for 24 hours at −5°. The charcoal was then collected by filtration and dried.

N-(*N*-Carbobenzoxy-L-seryl)-D-glucosamine (26 mg) was dissolved in a mixture of methanol and chloroform (5 ml) and the solution was added to water (5 ml) containing *N* hydrochloric acid (0.07 ml). Palladized charcoal was added and the mixture was shaken mechanically for 20 hours at room temperature under a slight pressure of hydrogen (2 lb above atmospheric pressure). The charcoal was removed from the product by filtration and the filtrate was concentrated to a syrup which was insoluble in chloroform and alcohol, but soluble in water.

The product was obtained from methanol/ether mixture as fine colorless crystals (19 mg; 86% yield) which decomposed over the temperature range 200–250°. On paper chromatograms developed in the 1-butanol/ethanol/water system the compound was revealed by the ninhydrin spray reagent as an orange spot which reddened on standing, and had $R_{GN\cdot HCl}$ 0.55. Anal. Found: N, 8.9; Cl, 11.7%. $C_9H_{19}O_7N_2Cl$ requires: N, 9.2; Cl, 11.7%.

The infrared spectrum showed a strong hydroxyl band at 3460 cm^{-1} which was very wide due to the amino group absorption. Amide I absorption was present at 1682 cm^{-1}, but was absent at 1495 cm^{-1}. Evidence for the ionic nitrogen was found at 1495 cm^{-1}.

The product prepared from the α-anomer failed to crystallize, and the yield of crude material was very poor.

(m) N-L-Seryl-D-glucosamine

Attempts to synthesize the free amine from *N*-(*N*-carbobenzoxy-L-seryl)-β-D-glucosamine by direct hydrogenolysis in aqueous solution failed, owing to the rapid decomposition of the product in the atmosphere. An indirect method was investigated. A little of the hydrochloride (*l*) (15 mg) was dissolved in an aqueous slurry of Amberlite IR 45 ($CO_3^=$) exchange resin (5 ml) and after removal of the resin the water was removed by distillation under reduced pressure at 30°, methanol being added to speed the evaporation of the water. The product which was a solid, blackened before it could be isolated.

(n) N-(N-Acetyl-O-acetyl-L-seryl)-1,3,4,6-tetra-O-acetyl-β-D-glucosamine

N-(L-Seryl hydrochloride)-β-D-glucosamine (10 mg) was acetylated with pyridine/acetic anhydride mixture (5 ml) at 0° for 2 days. The product was obtained as a syrup (10 mg) which had R_{GNpAc} 1.08 on paper chromatograms developed in the neutral solvent system. The infrared absorption spectrum of the product showed strong carbonyl of *O*-acetyl absorption at 1750 cm^{-1} but no band due to hydroxyl at 3460 cm^{-1}.

2. N-Glycyl-D-glucosamine

(a) N-Carbobenzoxyglycine

Glycine (25 g) was treated with carbobenzoxy chloride in toluene (60 g) using the method of Moore *et al.*, to give *N*-carbobenzoxyglycine (70 g).

(b) N-Carbobenzoxyglycyl p-Nitrophenyl Ester

N-Carbobenzoxyglycine (10 g) was dissolved in ethyl acetate (100 ml) containing *p*-nitrophenol (6.4 g) and dicyclohexycarbodiimide (9.2 g), and the mixture was kept at 0° for 1 hour and was then allowed to rise to room temperature. The precipitated dicyclohexyl urea by-product was removed by filtration and was washed free from the mother liquor with ethyl acetate. The combined filtrate and washings were concentrated and the ethanol solution of the residue gave the crystalline product (15 g; 95% yield) which had m.p. 127°. The infrared spectrum of the product showed the characteristic sharp absorption peaks due to the *p*-nitrophenyl group at 1775, 1635. 1600, 1540, 1500, 1355, and 695 cm^{-1}.

(c) N-(N-Carbobenzoxyglycyl)-1,3,4,6-tetra-O-acetyl-β-D-glucosamine

N-Carbobenzoxyglycyl *p*-nitrophenyl ester (1 g) was condensed with 1,3,4,6-tetra-*O*-acetyl-β-D-glucosamine hydrobromide (1.25 g) in the presence of dimethylformamide containing triethylamine (0.3 g) by Bodanszky and Du Vigneaud's (16) procedure to give the product, obtained as crystals (0.9 g) from methanol solution, which had m.p. 168°, and $[\alpha]_D^{22}$ +49° (*c*, 4.0 methanol).

The infrared spectrum of the product resembled that of the corresponding L-serine derivative (1*j*) showing absorption bands due to amide at 1675 cm^{-1} and carbonyl of *O*-acetyl at 1750 cm^{-1}. The "fingerprint" regions of the spectra were also similar, with peaks at 1375, 1250, 1058, and 695 cm^{-1}.

(d) N-(N-Carbobenzoxyglycyl)-D-glucosamine

N-(*N*-Carbobenzoxyglycyl)-1,3,4,6-tetra-*O*-acetyl-β-D-glucosamine (0.5 g) in dry methanol (10 ml) was treated with 0.4 *N* barium methylate (1 ml) at −5° for 20 hours and the deacetylated product was isolated as described for (1*k*), as crystals (0.2 g) which had m.p. 188° and $[\alpha]_D^{24}$ +39.5° (*c*, 4.0 methanol).

(e) N-(Glycyl hydrochloride)-D-glucosamine

N-(*N*-Carbobenzoxyglycyl)-D-glucosamine (0.15 g), dissolved in dioxan/water mixture (1:1 v/v) containing palladized charcoal (0.1 g), was placed under a slight pressure of hydrogen (2 lb above atmospheric pressure) and shaken for 24 hours. Hydrochloric acid (1 ml, 10%) was added to the mixture, the charcoal removed by filtration, and the filtrate was concentrated *in vacuo* to a syrup. The syrup was dissolved in hot ethanol from which the product was obtained as crystals (70 mg) which had m.p. 172° and $[\alpha]_D^{23}$ +31.5° (*c*, 1.0 water). Reported constants (8) for the product obtained by a different route are: m.p. 170° and $[\alpha]_D^{18}$ +29.7° (water).

The product was detected on paper chromatograms, developed in the neutral solvent system, by the ninhydrin and silver nitrate spray reagents as a single spot which had $R_{GN.HCl}$ 0.53.

(f) N-Glycyl-D-glucosamine

Attempts to remove the carbobenzoxy group from *N*-(*N*-carbobenzoxyglycyl)-1,3,4,6-tetra-*O*-acetyl-β-D-glucosamine and *N*-(*N*-carbobenzoxyglycyl)-D-glucosamine by hydrogenolysis in neutral solution, to form the free base, failed owing to the rapid decomposition of the product when it was exposed to the atmosphere. Attempts were made to deionize the salt (2*e*) with Amberlite IR 45($CO_3^=$) exchange resin but the product decomposed immediately on isolation.

3. N-L-Glutamyl-D-glucosamine

(a) N-Carbobenzoxy-L-glutamic Acid

L-Glutamic acid (25 g) was treated with 75% carbobenzoxychloride in toluene (44 ml) according to the procedure of Moore *et al.* (14), to give the crystalline product (51 g; 95% yield), which had m.p. 124°.

(b) N-Carbobenzoxy-L-glutamic Acid Di-p-nitrophenyl Ester

The ester was prepared by treating *N*-carbobenzoxy-L-glutamic acid (21 g) dissolved in ethyl acetate (200 ml) with *p*-nitrophenol (26 g) and dicyclohexylcarbodiimide (32 g) for 30 minutes at 0°. The reaction mixture was then kept at room temperature for 1 hour, the precipitated dicyclohexyl urea by-product was filtered off, and the filtrate was concentrated to a syrup which was dissolved in hot ethanol. On cooling, the crystalline product was obtained (21 g), which had m.p. 210° and $[\alpha]_D^{23}$ +17.1° (*c*, 4.0 benzene).

The infrared spectrum of the product showed no absorption due to hydroxy group, but showed bands due to the *p*-nitrophenyl group at 1775, 1540, 1500, 1355, and 1135 cm^{-1}.

(c) N-Carbobenzoxy-γ-L-glutamide α-p-Nitrophenyl Ester

The diester (3*b*) (10 g) dissolved in benzene/methanol mixture (100 ml; 1:1 v/v) was treated with the calculated amount of *N* methanolic ammonia, sufficient to react with one ester group, and the solution was allowed to stand overnight at room temperature. A small amount of precipitated material, identified as the diamide derivative, was removed and on the addition of benzene the crystalline monoamide was obtained (4.6 g; 60% yield) which after recrystallization from methanol had m.p. 158° and $[\alpha]_D^{21}$ −25.1° (*c*, 4.0 DMF). Anal. Found: N, 10.6%. $C_{19}H_{19}O_7N_3$ requires: N, 10.5%.

(d) 1-(1′,3′,4′,6′-Tetra-O-acetyl-β-D-glucosaminyl)-2-N-carbobenzoxy-L-glutamine

1,3,4,6-Tetra-*O*-acetyl-β-D-glucosamine hydrobromide (0.4 g) and the monoamide ester (3*c*) (0.4 g) dissolved in dimethylformamide (2 ml) were treated with excess triethylamine (0.2 g) at 95° for 20 minutes and the reaction mixture was then cooled to room temperature. Ether (4 ml) was added to precipitate the triethylamine hydrobromide which was removed by filtration, and the filtrate was then washed with *N* hydrochloric acid (3×20 ml) followed by water until the yellow color due to *p*-nitrophenol was removed. An ethanol solution of the product, diluted with chloroform, was dried (anhyd. sodium sulphate) and concentrated to a syrup which was dissolved in ethyl acetate and ether was added. The product was obtained as crystals (0.23 g) from the cooled solution which had, after recrystallization from ethanol, m.p. 178° and $[\alpha]_D^{20}$ −6.0° (*c*, 4.0 pyridine). The infrared spectrum of the product showed absorption bands in common with the D-glucosamine reactant at 1750, 1480, 1250, and 1050 cm^{-1}, and in common with the substituted amino acid at 3360, 1650, 1530, 1460, 1090, 1050, 900, and 695 cm^{-1}. The characteristic *p*-nitrophenyl bands were absent.

(e) 1-(1′,3′,4′,6′-Tetra-O-acetyl-β-D-glucosaminyl)-2-N-carbobenzoxy-L-glutamic Acid

The condensation product (3*d*) (50 mg) was treated with an excess of nitrous acid and when the reaction had subsided the solution was diluted with water and extracted with chloroform (3×10 ml). The dried (anhyd. sodium sulphate) chloroform extract was concentrated to a syrup (41 mg) which was purified by cellulose column partition chromatography using 1-butanol half saturated with water as the mobile phase. The final product (30 mg), obtained crystalline from ethanol solution, had m.p. 218° (decomp.) and $[\alpha]_D^{18}$ −7.4° (*c*, 2.0 pyridine).

(f) 1-(N-D-Glucosaminyl)-2-N-carbobenzoxy-L-glutamic Acid

The compound (3*e*) (30 mg) was treated with barium methoxide in the usual way to yield the deacetylated product (9 mg), which had m.p. 184°, and $[\alpha]_D^{18}$ +70° (*c*, 1.0 pyridine).

(g) 1-(N-D-Glucosaminyl)-L-glutamic Acid Hydrochloride

The deacetylated product (3*f*) (63 mg) dissolved in aqueous methanol (4 ml) containing 2 *N* hydrochloric acid (2 ml) was reduced with hydrogen in the presence of palladized charcoal as described for (1*l*). The product, crystallized from ethanol – light petroleum (b.p. 60–80°) mixture (30 mg), had $[\alpha]_D^{24}$ +14.1° (*c*, 1.5 water) and decomposed above 200°. Anal. Found: C, 37.9; H, 6.2; N, 8.0; Cl, 10.0%. $C_{11}H_{21}N_2O_8Cl$ requires: C, 38.3; H, 6.1; N, 8.1; Cl, 10.3%.

4. 1-O-β-L-Seryl-N-acetyl-D-glucosaminide

(a) 1-Chloro-1-deoxy-N-acetyl-3,4,6-tri-O-acetyl-α-D-glucosamine

The compound was prepared in 25% yield by the method of Inouye (18) and his co-workers. It had m.p. 124° and $[\alpha]_D^{25}$ +116° (*c*, 4.0 chloroform).

(b) N-Carbobenzoxy-L-seryl Methyl Ester

The methyl ester was synthesized by the action of diazomethane on the carboxylic acid (20). *N*-Carbobenzoxy-L-serine (8 g) dissolved in an alcohol/ether mixture (50 ml; 1:1 v/v) was treated with a solution of diazomethane (3 g) in ether (100 ml) and after the esterification was completed, the excess diazomethane was removed with hydrogen chloride gas. The solution was deacidified with Duolite A4(OH^-) exchange resin and then concentrated under reduced pressure to give the product as a pale yellow syrup (7 g).

(c) 1-O-β-(N-Carbobenzoxy-L-seryl methyl ester)-N-acetyl-D-glucosaminide

The substituted *O*-glucosaminide was synthesized by the Koenigs and Knorr (17) procedure, followed by deacetylation and hydrogenolysis of the product. Dried samples of 1-chloro-1-deoxy-*N*-acetyl-3,4,6-tri-*O*-acetyl-α-D-glucosamine (3.23 g) and *N*-carbobenzoxy-L-seryl methyl ester (2.5 g) were dissolved in anhydrous alcohol-free chloroform (50 ml) containing Drierite (10 g). Silver oxide (3 g) and iodine (5 mg catalyst) were added and the reaction mixture was shaken in the dark at room temperature for 140 hours. The insoluble material was removed by filtration through a Celite pad, the residue washed with chloroform, and the combined filtrate and washings were concentrated to a syrup. Paper chromatographic examination of the syrup in the neutral solvent system revealed the product as the major component having R_{GNAc} 3.2. The syrup was fractionated by cellulose column partition chromatography using 1-butanol half saturated with water as the mobile phase to give the chromatographically pure product (1.78 g) as a syrup which could not be crystallized. The product was deacetylated in absolute methanol (30 ml) containing 0.4 *N* barium methylate (2 ml) for 22 hours at −5°. The syrupy product (0.94 g) was found by paper chromatography to contain the required compound, which had R_{GNAc} 1.88, and *N*-acetylglucosamine. The faster-moving component was isolated by cellulose column partition chromatography using 1-butanol half saturated with water as the mobile phase. The product was crystallized from ethanol/light petroleum (b.p. 60–80°) mixture (219 mg). It had R_{GNAc} 1.89 and R_{GNpAc} 0.50, m.p. 133° and $[\alpha]_D^{23}$ +6.4° (*c*, 10 ethanol).

(d) 1-O-β-(L-Seryl methyl ester)-N-acetyl-D-glucosaminide

1-*O*-β-(*N*-Carbobenzoxy-L-seryl methyl ester)-*N*-acetyl-D-glucosaminide (140 mg) dissolved in aqueous ethanol (4 ml) containing palladized charcoal (200 mg), was placed under a slight pressure of hydrogen and shaken for 24 hours. The charcoal was removed by filtration and the filtrate was concentrated to a syrup (100 mg) which was shown by paper chromatography to contain the product contaminated with *N*-acetylglucosamine. The syrup was fractionated by chromatography on large sheets of Whatman 3MM paper in the neutral solvent system to give the pure derivative (60 mg) which had $R_{GN.HCl}$ 0.28, m.p. 230° (decomp.) and $[\alpha]_D^{24}$ +1.7° (*c*, 1.0 methanol).

The substituted D-glucosaminide was much more stable in air than the corresponding *N*-peptide.

(e) 1-O-β-(L-Seryl methyl ester hydrochloride)-N-acetyl-D-glucosaminide

1-*O*-β-(*N*-Carbobenzoxy-L-seryl methyl ester)-*N*-acetyl-D-glucosaminide (25 mg) was treated in the cold with 0.1 *N* hydrochloric acid (5 ml) for 6 hours, the excess acid was removed on Duolite A4(OH^-) exchange resin and the solution concentrated to dryness.

The product was twice recrystallized from ethanol/light petroleum (b.p. 60–80°) mixture. Anal. Found: C, 40.5; H, 6.7; N, 8.1; Cl, 9.8%. $C_{12}H_{22}N_2O_8Cl$ requires: C, 40.2; H, 6.4; N, 7.8; Cl, 9.90%.

The infrared spectrum of the product supported the assigned structure and showed absorption peaks at 1750 cm^{-1} due to ester carbonyl, 1610 cm^{-1} due to *N*-acetyl, and an ether-type absorption peak at 1100 cm^{-1}.

*(f) 1-O-β-*L*-Seryl-N-acetyl-*D*-glucosaminide*

The glucosaminide methyl ester (4*d*) (48 mg) dissolved in methanol (0.5 ml) was diluted with water (2 ml) and 0.5 *N* sodium hydroxide solution (3 ml) was slowly added and the saponification was allowed to proceed for 3 hours at room temperature. The solution was made slightly acidic by the addition of 0.1 *N* sulphuric acid (0.24 ml) and the solution was concentrated under reduced pressure. The residue was extracted with methanol from ionic material and the concentrated extract was dissolved in ethanol/light petroleum (b.p. 60–80°) mixture which on cooling gave the crystalline product (27 mg) which had m.p. 236°, and $[\alpha]_D^{24}$ −31.0° (*c*, 1.0 water). Anal. Found: C, 42.85; H, 6.4; N, 9.0%. $C_{11}H_{20}N_2O_8$ requires: C, 42.9; H, 6.5; N, 9.1%.

The infrared absorption spectrum of the product showed no absorption peak due to ester group at 1750 cm^{-1} but the strong absorption band at 1610 cm^{-1} due to *N*-acetyl was present.

DISCUSSION

The object of this investigation was to determine the properties of D-glucosamine derivatives of some amino acids, particularly their stability in solution.

The prepared derivatives were dissolved in acidic media in order to investigate their behavior under conditions of acid hydrolysis. The *N*-peptide hydrochlorides were found to be unaffected by *N* hydrochloric acid at 60° for 24 hours, but the 1-*O*-β-L-seryl-D-glucosaminide gave L-serine, *N*-acetyl-D-glucosamine, and D-glucosamine after 1 hour under the same conditions.

The *N*-peptides were stable in neutral or basic media but degraded rapidly in the presence of air, and immediately upon isolation. It is apparent that the *N*-peptides may only be obtained as their salts, from acid media, whereas the D-glucosaminides examined are relatively unstable in acid solution and should be separated only from neutral or basic media.

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GUANIDINE COMPOUNDS

IV. ACETYLATION OF SOME ALKYL-SUBSTITUTED GUANIDINES WITH ACETIC ANHYDRIDE AND ETHYL ACETATE[1,2]

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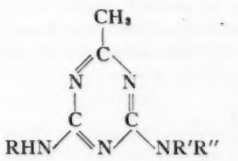
ABSTRACT

The acetylation of some mono-, N,N-, and N,N′-dialkylguanidines was studied using ethyl acetate and acetic anhydride. Except for the N,N-dialkylguanidines, the action of ethyl acetate on the free bases gave monoacetyl derivatives. Acetic anhydride with the acetate salts gave diacetyl derivatives with monoalkylguanidines, acetamido-1,3,5-triazines with N,N-dialkylguanidines, and unstable di- and tri-acetyl derivatives with N,N′-dialkylguanidines. The assignment of symmetrical structures to the mono- and tri-acetyl derivatives and an asymmetrical structure to the diacetyl derivatives was supported by nuclear magnetic resonance spectra. The ultraviolet spectra of the monoacetyl derivatives exhibited one peak in the region 230–235 mμ whilst the diacetyl derivatives showed two peaks in the regions 220–230 mμ and 253–263 mμ. Some comments are made on the infrared spectra of these compounds.

In an earlier paper in this series Cockburn and Bannard (1) reported the formation of amino- (I) and acetamido-*s*-triazines (II) on acetylation of alkylguanidine salts under vigorous conditions.



I. R = H; R′, R″ = H or alkyl
II. R = CH_3CO; R′, R″ = H or alkyl
III. R = CH_3CO; R′, R″ = CH_3

Ryabinin (2) had previously shown that the products obtained on acetylation of guanidine acetate were dependent on the conditions used. Under mild conditions, an acetylated guanidine derivative was obtained rather than an *s*-triazine derivative. The present study, in which the acetylation of several mono-, N,N-, and N,N′-dialkylguanidines with ethyl acetate and acetic anhydride was examined, revealed that the type of alkyl substitution also influenced the product obtained. The opportunity was taken to examine the infrared and ultraviolet spectra of the acetylated guanidines isolated.

Various conditions for the acetylation of *trans*-2-guanidocyclohexanol with acetic anhydride were examined to determine which gave the highest yield of acetylated products. The addition of sodium acetate did not influence the yields and was therefore omitted, since it merely complicated isolation of the products. It was found that a shorter time of heating at 100° than that previously employed for the acetylation of guanidine (3) was beneficial, since even the relatively stable diacetyl derivatives were degraded or cyclized on prolonged heating with acetic anhydride at this temperature.

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[2]*Part III, Can. J. Chem.* **37**, *1810 (1959).*

The use of the readily soluble acetate salts rather than the more insoluble hydrochlorides, nitrates, or sulphates enabled a reaction period of 30 minutes to be used.

Acetylation of the monoalkylguanidines under these conditions gave the diacetyl derivatives in yields exceeding 79%, together with small amounts of the corresponding acetylated amino compounds. The latter products were shown to result from degradation of the diacetyl derivatives by acetic anhydride, since on warming of diacetyl cyclohexylguanidine for 1 hour at 100° with acetic anhydride, acetyl cyclohexylamine was isolated in 50% yield.

The N,N-dialkylguanidines gave, in general, acetamido-*s*-triazines, although in the case of N,N-dimethylguanidine a diacetyl derivative was also isolated. The results given in Table I show that the amount of diacetyl derivative (IV) decreases as the amount of acetamido-*s*-triazine (III) increases when the heating period is prolonged. It thus appears that diacetyl derivatives are intermediates in the formation of triazines from N,N-dialkylguanidines. This behavior contrasts with that of the diacetyl derivatives of the monoalkylguanidines, which, on being heated with acetic anhydride, are degraded rather than cyclized. The formation of triazines from monoalkylguanidine acetates or monoacetyl derivatives has been reported (4) but more drastic conditions were involved (0.5 hour at 200°).

TABLE I

Variation of product composition on heating N,N-dimethylguanidine with acetic anhydride at 100° for various periods of time

Heating period (min)	Percentage yield of products	
	III	IV
15	24	46
30	32	31
60	49	23

NOTE: III = 2-dimethylamino-4-acetamido-6-methyl-1,3,5-triazine. IV = diacetyl N,N-dimethylguanidine.

With N,N′-dialkylguanidines, the products obtained were very dependent on the individual structure of the guanidine. Both six-membered ring compounds gave unstable diacetyl derivatives which could only be isolated by vacuum distillation. Attempts to purify them by chromatography on alumina, or recrystallization from alcohol gave the more stable monoacetyl derivatives. N,N′-Dimethylguanidine together with 2-iminoimidazolidine gave compounds whose analysis indicated a triacetyl derivative. Because of the peculiar spectral and chemical properties of triacetyl 2-iminoimidazolidine it is discussed more fully later in the paper.

Acetylation of alkylguanidines with ethyl acetate was studied with the object of preparing monoacetyl derivatives. Ostrogovich (5) reported the acetylation of aminotriazines by boiling them with ethyl acetate or acetic acid. The reaction most likely occurs by nucleophilic attack of the π electrons of the guanidine on the ester, similar to the mode of action formulated by Gordon *et al.* (6) for the ammonolysis of esters. Simons and Weaver (4) showed that ethyl acetate reacts with monoalkylguanidine free bases in the cold to give the monoacetyl derivatives. We modified their procedure by using Amberlite IRA-400 (OH) resin prepared in absolute alcohol to generate the free base. This procedure had the advantage over the usual one in which the hydrochloride

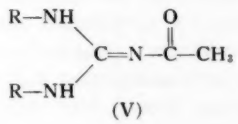
is treated with sodium ethoxide in that it gave quantitative conversion to the pure free base, uncontaminated with inorganic material. The type of alkyl substituents again affected the type of product obtained. With the exception of the N,N-dialkylguanidines all the alkylguanidines gave monoacetyl derivatives by this method. Since Angyal and Warburton (7) and Neivelt *et al.* (8) have shown that methyl guanidines, regardless of the type and degree of alkyl substitution have a pK_a greater than 13.4, basicity is obviously not the reason for lack of reactivity. It is interesting to note that 2-imino-5-hydroxyhexahydropyrimidine gave only a mono-N-acetyl derivative, indicating that the method could be used to selectively monoacetylate a guanidine moiety in a molecule also possessing a hydroxyl function. Acetylation of guanidine itself gave a monoacetyl derivative which is still basic (pK_a 8.2) but would not form a diacetyl derivative on further treatment with ethyl acetate.

TABLE II

Ultraviolet absorption of monoacetyl guanidines in absolute alcohol

Compound	λ_{max} (mμ)	log ϵ_{max}
Acetyl guanidine	230	4.24
Acetyl methylguanidine	234	4.31
Acetyl cyclohexylguanidine	232	4.30
N-Acetyl guanidocyclohexanol	234	4.26
Acetyl N,N′-dimethylguanidine	234	4.23
Acetyl 2-iminoimidazolidine	230	4.24
Acetyl 2-iminohexahydropyrimidine	234	4.23
Acetyl 2-imino-5-hydroxyhexahydropyrimidine	235	4.31
Acetyl guanidine acetate	230	3.92

The ultraviolet spectra of the monoacetyl guanidines, when measured in ethanol, all exhibit one peak in the range 230–235 mμ as shown in Table II. This absorption is independent of the type of alkyl substitution possessed by the guanidine moiety. A similar observation was reported by McKay *et al.* (9) for alkyl nitroguanidines.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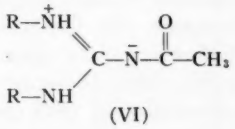
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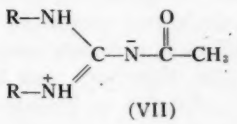
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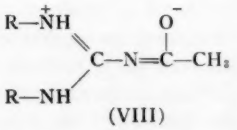
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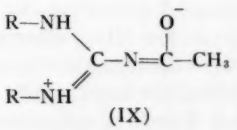

(IX)

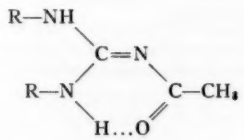
Since neither guanidine nor primary or secondary amides absorb strongly above 210 mμ, the absorption indicates conjugation of the acetyl carbonyl group with the guanidine moiety. It was therefore concluded that monoacetyl guanidines exist in the acetylimino form (V, R = alkyl or H) and this was confirmed in the case of acetyl 2-iminoimidazolidine by measurement of its nuclear magnetic resonance spectrum.

Some contribution to the structure from the other canonical forms of the guanidine moiety (VI) and (VII), together with a small contribution from forms (VIII) and (IX)

would also be expected. Bryden *et al.* (10), from crystallographic studies, showed this to be the case for nitroguanidine, which is best formulated as a resonance hybrid which combines both the forms postulated by McKay *et al.* (9) and by Kirkwood and Wright (11).

The infrared spectra of monoacetyl guanidines would be expected to show two bands in the region 1700–1500 cm^{-1} corresponding to the stretching vibrations of the C=N and the C=O of the acetyl group (amide I (12)). Table III shows the results obtained.

The spectra mainly show one very strong broad band at 1620–1580 cm^{-1} with shoulders on the high and low frequency sides. This strong band is presumably due to the unresolved vibrations of the C=N and C=O groups. Two bands are observed only in the cases of the monoacetyl derivatives of 2-iminoimidazolidine and 2-imino-5-acetoxyhexahydropyrimidine. The stronger band, at 1580 cm^{-1} and 1595 cm^{-1} respectively, was assigned to amide I vibrations, since it is doubtful whether the intensity of the C=N vibration, even though it is known to increase on conjugation (13), would approach that of an amide I. Also, the amide I is known to be more susceptible to displacement than the C=N vibration. The large shift found for the amide I band can be attributed partly to intramolecular hydrogen bonding as shown in X and partly to conjugation. Examination of the spectrum of triacetyl 2-iminoimidazolidine, a compound which contains no NH bond shows a normal amide I vibration with no absorption around 1600 cm^{-1}.



(X)

The second band found in the spectra of both monoacetyl 2-iminoimidazolidine and monoacetyl 2-imino-5-acetoxyhexahydropyrimidine occurs at 1620 cm^{-1} and is assigned to the C=N stretching vibration, the shift to lower frequency resulting from conjugation with the acetyl group. In the literature, the C=N vibration for substituted nitroguanidines is variously assigned, Leiber *et al.* (14) give 1680–1661 cm^{-1}, Randall *et al.* (15) 1695–1639 cm^{-1}, and Kumler (16) 1689–1618 cm^{-1}. Kumler's assignment for the C=N vibration in 2-nitriminoimidazolidine at 1618 cm^{-1} agrees closely with that of the acetyl analogue, but his assignment of a band at 1664 cm^{-1} in nitroguanidine itself seemed high. Monoacetyl guanidine also shows a band at 1660 cm^{-1} which we thought was most likely due to the NH_2 deformation vibrations as suggested by Jones (17) for guanidine. Attempts to deuterate acetyl guanidine resulted in hydrolysis, but deuteration of nitroguanidine caused the band at 1664 cm^{-1} to disappear whilst another band appeared at 1290 cm^{-1}. Hence Kumler's assignment of the 1664 cm^{-1} band in nitroguanidine to the C=N appears to be in error and it must appear at lower frequencies together with the vibration for the nitro group.

The diacetyl guanidines possess characteristic ultraviolet spectra by means of which they can be readily distinguished from mono- or tri-acetyl guanidines. All the diacetyl guanidines show two peaks in the regions 212–225 mμ and 243–263 mμ as shown in Table V.

In all cases examined the extinction coefficient of the low wavelength peak was just

TABLE III

Infrared absorption of some monoacetyl guanidines in the region 1700–1550 cm^{-1}

Compound	Absorption (cm^{-1})				Type of spectrum
Acetyl guanidine	1660 (m)	1605 (sh)	1600 (vs)	—	KBr pellet
Acetyl cyclohexylguanidine	—	1615 (sh)	1590 (vs)	1565 (sh)	KBr pellet
	—	1640 (sh)	1595 (vs)	1575 (sh)	Chloroform solution
Acetyl *trans*-2-guanidocyclohexanol	—	1640 (sh)	1600 (vs)	1580 (sh)	KBr pellet
Acetyl 2-iminohexahydropyrimidine	—	1620 (sh)	1595 (vs)	1560 (sh)	KBr pellet
	—	1620 (sh)	1595 (vs)	1570 (sh)	Chloroform solution
Acetyl 2-iminoimidazolidine	1660 (w)	1620 (sh)	1595 (vs)	1565 (sh)	KBr pellet
	—	1620 (s)	1580 (vs)	—	Chloroform solution
Acetyl 2-imino-5-acetoxyhexahydropyrimidine	—	1620 (s)	1595 (vs)	1575 (sh)	KBr pellet

TABLE IV

Infrared absorption of some diacetyl guanidines in the region 1750–1550 cm^{-1}

Compound	Absorption frequencies (cm^{-1})					Type of spectrum
Diacetyl guanidine	—	1700 (s)	1625 (s)	1585 (vs)	1550 (m)	KBr pellet
Triacetyl *trans*-2-guanidinocyclohexanol	1740 (s)	1705 (s)	1620 (sh)	1595 (vs)	1570 (m)	KBr pellet
Diacetyl cyclohexylguanidine	—	1700 (s)	1615 (sh)	1600 (vs)	1565 (m)	KBr pellet
Diacetyl N,N-dimethylguanidine	—	1705 (m)	1620 (sh)	1595 (vs)	1550 (m)	KBr pellet
Diacetyl 2-iminohexahydropyrimidine	—	1670 (m)	—	1595 (s)	—	KBr pellet
	—	1680 (m)	—	1605 (s)	—	Chloroform solution
Diacetyl 2-imino-5-acetoxyhexahydropyrimidine	1725 (s)	1680 (m)	1620 (m)	1595 (vs)	—	KBr pellet

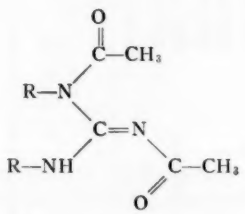
TABLE V

Ultraviolet absorption of diacetyl guanidines

Compound	λ_{max} (mμ)	log ϵ_{max}	λ_{max} (mμ)	log ϵ_{max}	Solvent
Diacetyl guanidine	212	3.95	243	4.25	Abs. ethanol
Diacetyl guanidocyclohexanol	222	3.99	255	4.23	Abs. ethanol
Diacetyl cyclohexylguanidine	221	4.03	254	4.25	Abs. ethanol
Diacetyl methylguanidine	218	4.01	254	4.24	Cyclohexane
Diacetyl N,N′-dimethylguanidine	225	3.87	263	4.29	Abs. ethanol
Octa-acetyl streptidine	220	4.27 (3.99)*	254	4.55 (4.25)*	Abs. ethanol
Diacetyl 2-iminohexahydropyrimidine	223	3.95	256	4.24	Cyclohexane
Diacetyl 2-imino-5-acetoxyhexahydropyrimidine	225	3.97	254	4.17	Cyclohexane

*Log ϵ_{max}/guanidine moiety.

about half that for the peak at higher wavelength. Unlike monoacetyl guanidines, alkyl substitution does affect the chromophore for the diacetyl guanidines, resulting in a variation of 13 mμ for the first peak and 20 mμ for the second. The bathochromic shift observed in the ultraviolet spectra in going from mono- to di-acetyl derivatives is compatible with a further lengthening of the π electron path. An asymmetrical structure XI was assigned to the diacetyl derivatives based on nuclear magnetic resonance studies with the diacetyl derivative of 2-iminohexahydropyrimidine.



(XI)

Korndorfer (18) postulated the existence of both symmetrically and asymmetrically substituted diacetyl guanidines. Later Ryabinin (2) established that only one isomer exists, but did not specify which one. The infrared spectra of the diacetyl guanidines also give ample evidence for the asymmetric structure, the results being given in Table IV.

The spectra exhibit a very strong broad band in the region 1620–1585 cm^{-1} together with a strong band in the region 1705–1670 cm^{-1}. The first band is similar to that exhibited by the monoacetyl guanidines and, as in that case, is ascribed to the unresolved stretching frequencies of the C=N and the C=O of the acetylimino group. The second band is assigned to the amide I vibration of the second acetyl group by analogy with the absorption observed for the acetamido group in 6-methyl-2-cyclohexylamino-4-acetamido-1,3,5-triazine at 1680 cm^{-1}, 6-methyl-2-dimethylamino-4-acetamido-1,3,5-triazine at 1685 cm^{-1}, and 6-methyl-2-piperidino-4-acetamido-1,3,5-triazine at 1690 cm^{-1}. The shift to higher frequencies of the amide I band of the second acetyl group is expected, since Richards and Thompson (19) showed that substitution of an amide nitrogen with an electrophilic group produces this effect. We found the amide I band above 1700 cm^{-1}

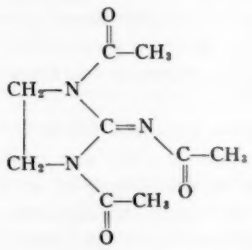
if the second acetyl group is present as a secondary amide and below if present as a tertiary amide, e.g. diacetyl N,N-dimethylguanidine absorbs at 1705 cm^{-1} (KBr) and diacetyl hexahydropyrimidine at 1670 cm^{-1} (KBr). Since the main difference between the amides is in the nature of the third group attached to the amide nitrogen, i.e. either a hydrogen or an alkyl group, the spectral data can be explained in terms of the electron-donating power of the third group. The alkyl group is able to contribute a greater share of its electrons to the amide nitrogen than the hydrogen, thus reducing the demand placed on the nitrogen by the acetyl and acetylimino groups. Hence the increase in frequency is not as great for the tertiary amide. In diacetyl guanidines possessing a secondary amide group, a third band in the range 1575–1560 cm^{-1} is found, due to the amide II vibration.

The ultraviolet spectra of the triacetyl derivatives obtained from N,N′-dimethylguanidine and 2-iminoimidazolidine were similar, showing only one peak at 232 $m\mu$ and 234 $m\mu$ respectively. This absorption is similar to that obtained with the monoacetyl derivatives, although it seems unlikely that the same chromophore is involved. The infrared spectra showed no absorption above 3000 cm^{-1} or near 1600 cm^{-1} in either case, and this was interpreted as evidence for intramolecular hydrogen bonding between the N—H and acetylimino groups in mono- and di-acetyl guanidines. The spectra differed in that triacetyl N,N′-dimethylguanidine shows two strong bands at 1690 and 1620 cm^{-1} (film), whereas triacetyl 2-iminoimidazolidine shows three in chloroform solution at 1745, 1690, and 1645 cm^{-1} and four for a KBr pellet at 1725, 1690, 1680, and 1620 cm^{-1}. The band at lowest frequency is thought to be due to the amide I of the non-hydrogen-bonded acetylimino group, whilst bands at 1690 cm^{-1} are due to the amide I of the tertiary amides. The band found at 1745 cm^{-1} (chloroform) and 1725 cm^{-1} (KBr) in triacetyl 2-iminoimidazolidine appears to be associated with the C=N, since it is missing from the methanol adduct of triacetyl 2-iminoimidazolidine where addition has taken place across the C=N bond. Since it is also missing from triacetyl N,N′-dimethylguanidine, it appears to be associated with the cyclic structure. The shift in the amide I frequently observed for the lactam group in ethylene urea from 1652 cm^{-1} to 1752 cm^{-1} (KBr) in the diacetyl ethylene urea lends credence to this idea.

The proof that triacetyl 2-iminoimidazolidine has a symmetrical structure (XII) was obtained from its nuclear magnetic resonance spectrum, which showed three peaks at 3.41, 4.96, and 5.12 p.p.m. upfield from chloroform with relative intensities 4:6:3. The first peak was assigned to the methylene hydrogens and as no splitting was apparent, it was taken as evidence that they were equivalent. The two remaining peaks were assigned to the methyl hydrogens of the acetyl groups, the intensities indicating that two of the three methyl groups were similar. Formation of an addition product on warming of triacetyl 2-iminoimidazolidine with alcohol is of interest in view of the fact that dinitro-2-iminoimidazolidine also exhibits such a property with alcohols and amines (20). The cyclic structure (XIII) was assigned to it on the basis of its analysis and infrared spectrum. The presence of bands at 3142(m), 1650(s), and 1525(m) cm^{-1} (KBr pellet) was taken as indicative of the formation of a secondary amide. The absence of the C=N group was indicated by the stability of the addition product to boiling water, since under these conditions triacetyl 2-iminoimidazolidine readily yields diacetyl ethylene urea.

To summarize, this work has shown that the products obtained on acetylation of alkylguanidines depend on the degree and position of alkyl substitution, as well as the

conditions used. Analogies have been shown to exist between the chemistry of the nitro and acetyl derivatives of alkylguanidines. This is to be expected, since a similarity does exist between the inductive and mesomeric effects of the two groups, although it is of a qualitative nature. However, the extent to which a guanidine moiety is nitrated or acetylated is not necessarily the same, e.g. 2-iminoimidazolidine forms a dinitro (20) but a triacetyl derivative.



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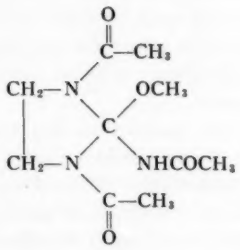

(XIII)

The difference in reactivity of the five- and six-membered cyclic N,N′-dialkylguanidines on acetylation must be attributed to the effect of the geometry of the ring on the guanidine moiety, since no difference exists in the basicity of these guanidines.

EXPERIMENTAL

All melting points are uncorrected unless otherwise stated. Microanalyses are by Micro-Tech Laboratories, Skokie, Ill., and J. Helie of these laboratories. Ultraviolet spectra were measured on a Cary Model 14 P.M. recording spectrophotometer, while the infrared spectra were measured on a Baird double beam recording spectrophotometer.

Starting Materials

Methylguanidine was purchased from Mann Research Laboratories. Cyclohexylguanidine, 2-guanidocyclohexanol, N,N-dimethylguanidine, guanylpyrrolidine, and guanylpiperidine were prepared from the corresponding amines by the action of 1-guanyl-3,5-dimethylpyrazole nitrate as described by Bannard *et al.* (21). The method of Mold *et al.* (22), employing cyanogen bromide and methylamine, was used to prepare N,N′-dimethylguanidine. Synthesis of 2-iminoimidazolidine, 2-iminohexahydropyrimidine, and 2-imino-5-hydroxyhexahydropyrimidine involved first preparation of the corresponding cyclic nitrimines as described by McKay and Wright (23), followed by treatment of the nitrimines with liquid ammonia as reported by Stefanye and Howard (24). We isolated these compounds as the acetate salts, which are described in Table VI.

All the other alkylguanidines were converted into the acetate salts by ion exchange and dried prior to acetylation with acetic anhydride.

Acetylation of Guanidine Free Bases with Ethyl Acetate

An Amberlite IRA-400 (OH^-) resin column (15 ml) was prepared in the usual manner, then washed for 24 hours with absolute ethanol excluding carbon dioxide. Guanidine hydrochloride (500 mg, 5.2 millimoles) was dissolved in absolute ethanol (15 ml) and the solution passed through the column, after which the column was washed with absolute ethanol, a total of 100 ml eluate being collected. Evaporation of the latter to dryness

TABLE VI

Cyclic guanidine acetates

Compound	Formula	m.p.	C		H		N	
			Calc.	Found	Calc.	Found	Calc.	Found
2-Iminoimidazolidine acetate	$C_5H_{11}O_2N_3$	186–188°	41.37	41.51	7.64	7.81	28.95	28.66
2-Iminohexahydropyrimidine acetate	$C_6H_{13}O_2N_3$	141–143°	45.27	45.32	8.37	8.30	26.30	26.17
2-Imino-5-hydroxyhexahydropyrimidine acetate	$C_6H_{13}O_3N_3$	134–135°	41.13	40.90	7.48	7.65	23.99	23.91

TABLE VII

Monoacetyl guanidines

Compound	Formula	m.p.	C		H		N		Yield (%)
			Calc.	Found	Calc.	Found	Calc.	Found	
Acetyl guanidine	$C_3H_7ON_3$	188–190°	35.63	35.62	6.95	7.07	41.56	41.56	89
Acetyl methylguanidine	$C_4H_9ON_3$	158–160°	41.72	41.45	7.88	7.62	36.50	36.63	73
Acetyl 2-iminoimidazolidine	$C_5H_9ON_3$	227–229°	47.23	47.05	7.13	7.11	33.05	32.93	84
Acetyl cyclohexylguanidine	$C_9H_{17}ON_3$	163–165°	59.98	59.96	9.35	9.28	22.93	23.10	92
Acetyl 2-guanidinocyclohexanol	$C_9H_{17}O_2N_3$	183–185°	54.25	54.34	8.60	8.57	21.09	21.26	80
Acetyl 2-imino-5-hydroxyhexahydropyrimidine	$C_6H_{11}O_2N_3$	213–215°	45.85	45.84	7.05	7.25	26.74	26.82	87
Acetyl 2-iminohexahydropyrimidine	$C_6H_{11}ON_3$	189–191°	51.04	51.07	7.85	8.00	29.77	29.55	71

in vacuo gave a colorless oil which was dissolved in absolute alcohol (0.5 ml), to which solution freshly distilled ethyl acetate (0.5 ml, 5.2 millimoles) was slowly added. Although colorless crystals formed instantly, the solution was allowed to stand overnight at room temperature before they were filtered off, washed with ethyl acetate/ethanol and finally recrystallized from ethanol/acetone to give 475 mg (89%) of monoacetyl guanidine as colorless prisms, m.p. 188–190°. The compound was characterized by its acetate, m.p. 178–180°, lit. 180° (3), picrate m.p. 237–239°, lit. 238° (2).

All the other monoacetyl derivatives shown in Table VII were prepared in a similar manner.

Acetylation of Guanidine Acetate Salts with Acetic Anhydride

Cyclohexylguanidine

Cyclohexylguanidine hydrochloride (500 mg) was converted to the acetate salt by ion exchange using Amberlite IRA-400 (OAc) resin. The acetate salt was thoroughly dried *in vacuo* (0.05 mm) at room temperature before addition of acetic anhydride (5 ml). The mixture was heated at 100° for 1/2 hour, after which the acetic anhydride was removed *in vacuo* leaving 493 mg of brown oil. The oil was chromatographed on Woelm IV neutral alumina and gave two fractions. Elution with 2:1 hexane/benzene yielded a crystalline material, which, after recrystallization from pentane, gave colorless prisms, m.p. 71–72°, and analyzed for diacetyl cyclohexylguanidine. Calc. for $C_{11}H_{19}O_2N_3$: C, 58.64; H, 8.50; N, 18.65. Found: C, 58.87; H, 8.64; N, 18.36%. Yield: 445 mg (86%). Further elution with ether gave more crystalline material which was recrystallized from ether/pentane yielding fine white needles, m.p. 106–107°, lit. 107° (25), yield 37 mg (10%). Analysis and melting point indicated it to be N-acetyl cyclohexylamine, and this was confirmed by preparation of an authentic sample and comparison of the infrared spectra.

2-Guanidocyclohexanol

2-Guanidocyclohexanol sulphate (500 mg) was acetylated as described above yielding 530 mg of light brown oil which gave two products on chromatography. The first was obtained by elution with 2:1 hexane/benzene, and after recrystallization from pentane yielded colorless crystals, m.p. 69–70°, which analyzed for a triacetyl derivative, the infrared spectrum indicating O,N,N′ substitution. Calc. for $C_{13}H_{21}O_4N_3$: C, 55.11; H, 7.47; N, 14.83. Found: C, 55.04; H, 7.53; N, 14.53%. Yield: 498 mg (79%). The second product which was eluted with 1:1 ether/chloroform, appeared from analysis, infrared spectrum, and m.p. 117–118°, lit. 117° (26), to be O,N-diacetyl *trans*-2-aminocyclohexanol. Yield: 26 mg (5%). Verification was obtained by synthesis of the compound and comparison of the infrared spectra.

N,N-Dimethylguanidine

N,N-Dimethylguanidine sulphate (475 mg) was acetylated as above and the crude material chromatographed on neutral alumina yielding two products. The first product was obtained by elution with 1:1 pentane/benzene and after recrystallization from benzene/ether gave colorless prisms, m.p. 115–117°, which analyzed for 4-acetamido-2-dimethylamino-6-methyl-1,3,5-triazine. Calc. for $C_8H_{13}ON_5$: C, 49.22; H, 6.77; N, 35.88. Found: C, 49.44; H, 6.83; N, 35.83%. Yield: 77 mg (32%). The infrared spectrum was in agreement with this assignment. A second substance was obtained by elution with 2:1 chloroform/ether which gave colorless prisms after recrystallization from methanol/ether, m.p. 176–178°, and analyzed for diacetyl N,N-dimethylguanidine. Calc. for

$C_8H_{13}O_2N_5$: C, 49.11; H, 7.65; N, 24.55. Found: C, 49.24; H, 7.74; N, 24.90%. Yield: 92 mg (31%).

2-Iminoimidazolidine

2-Iminoimidazolidine hydrochloride (500 mg) was acetylated as above and the oily product remaining after removal of the acetic anhydride slowly crystallized. Further recrystallization from acetone gave long white needles, m.p. 141–142°, which, from its analysis, appeared to be triacetyl 2-iminoimidazolidine. Calc. for $C_9H_{13}O_3N_3$: C, 51.17; H, 6.20; N, 19.9; CH_3CO, 61.13. Found: C, 51.25; H, 6.33; N, 19.85; CH_3CO, 60.82%. Yield: 441 mg (51%).

Warming the triacetyl derivative with methanol for 20 minutes at 50° gave a quantitative yield of the methanol adduct, which was recrystallized from the same solvent, giving white rhombohedral crystals, m.p. 165–167°. Calc. for $C_{10}H_{17}O_4N_3$: C, 49.39; H, 7.04; N, 17.28; CH_3CO, 53.60; CH_3O, 12.77. Found: C, 49.56; H, 7.00; N, 17.06; CH_3CO, 53.63; CH_3O, 12.43%.

2-Iminohexahydropyrimidine

2-Iminohexahydropyrimidine acetate (500 mg) on acetylation under the usual conditions gave an oily product which distilled at 105–110° at 0.01 mm, giving a clear oil which slowly crystallized. Recrystallization from pentane/ether gave colorless thick prisms, m.p. 61–63°, which after drying *in vacuo* analyzed for diacetyl 2-iminohexahydropyrimidine. Calc. for $C_8H_{13}O_2N_3$: C, 52.44; H, 7.15; N, 22.94. Found: C, 52.70; H, 6.91; N, 22.61%. Yield: 371 mg (65%).

Attempts to chromatograph the crude product on neutral alumina or to recrystallize the diacetyl derivative from solvents containing alcohol led to the isolation of monoacetyl 2-iminohexahydropyrimidine, m.p. 187–189°, identified by its infrared spectrum.

2-Imino-5-hydroxyhexahydropyrimidine

The acetate salt (500 mg) was acetylated in the usual manner giving an oily product which distilled at 115–125° at 0.01 mm. The oil crystallized from pentane/ether giving fine white needles, m.p. 117–119°, which analyzed for a triacetyl derivative, with the infrared spectrum indicating O,N,N′ substitution. Calc. for $C_{10}H_{15}O_4N_3$: C, 49.78; H, 6.27; N, 17.42. Found: C, 49.49; H, 6.92; N, 17.62%. Yield: 325 mg (57%).

Recrystallization of the triacetyl derivative from solvents containing alcohol, or chromatography on alumina, caused decomposition. The resulting product could be purified either by sublimation *in vacuo* at 140–150° at 0.01 mm or by recrystallization from methanol/ether, which gave white microprisms, m.p. 230–232°, analyzing for a diacetyl derivative. The infrared spectrum indicated the compound to be the O,N-diacetyl derivative. Calc. for $C_8H_{13}O_3N_3$: C, 48.23; H, 6.58; N, 21.18. Found: C, 48.28; H, 6.53; N, 21.40%.

Methylguanidine

Methylguanidine acetate (500 mg) was acetylated at 100° for only 15 minutes, and the oily product was chromatographed on neutral alumina. Elution with benzene, followed by recrystallization from ether/pentane furnished colorless thick needles, m.p. 80–82°, analysis of which indicated diacetyl methylguanidine. Calc. for $C_6H_{11}O_2N_3$: C, 45.85; H, 7.05; N, 26.74. Found: C, 46.15; H, 6.91; N, 26.99%. Yield: 289 mg (49%). Further elution with 1:1 chloroform/benzene gave a solid, which, after recrystallization from methanol/ether, had m.p. 158–160° and was identified as monoacetyl methylguanidine by comparison of infrared spectra. Yield: 53 mg (12%).

Guanylpiperidine

Guanylpiperidine hydrochloride (500 mg) was converted to the acetate salt by ion exchange, after which it was acetylated with excess acetic anhydride for 30 minutes at 100°. The resulting yellowish oil was chromatographed on Woelm IV neutral alumina yielding only one product which was eluted with 1:1 pentane/ether and gave colorless crystals, m.p. 147–148°, from acetone/hexane. Comparison of its infrared spectrum with that of an authentic sample (1) together with its analysis proved it to be 4-acetamido-6-methyl-2-piperidino-1,3,5-triazine. Yield: 202 mg (56%).

Guanylpyrrolidine

Guanylpyrrolidine acetate (500 mg) was acetylated as described above producing a dark red oil together with some crystals. Treatment with cold acetone removed the oil leaving yellow needle-like crystals, which after recrystallization from methanol/ether had m.p. 173–175° and analyzed for 4-acetamido-6-methyl-2-pyrrolidino-1,3,5-triazine. Calc. for $C_{10}H_{15}ON_5$: C, 54.28; H, 6.83; N, 31.66. Found: C, 54.40; H, 6.25; N, 31.38%. Yield: 203 mg (67%).

N,N'-Dimethylguanidine

Dimethylguanidine acetate (500 mg) was acetylated in the usual manner, giving a clear oily product. Chromatography on neutral alumina caused decomposition so the product was purified by distillation at 100–110° at 0.05 mm, giving a colorless oil which analyzed for the triacetyl derivative. Calc. for $C_9H_{15}O_3N_3$: C, 50.69; H, 7.09; N, 19.71. Found: C, 50.39; H, 7.32; N, 19.92%. (λ_{max} 234 mμ, log ϵ 4.2.) Yield: 444 mg (61%).

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CO-ORDINATION COMPOUNDS OF NICKEL (II)

PART II. NEW SPECIES OF NICKEL (II) – SALICYLALDIMINE COMPLEXES[1]

H. C. CLARK AND R. J. O'BRIEN

ABSTRACT

The preparation of two solid paramagnetic forms, one light green and the other buff-colored, of bis-N-methyl-5-chloro-salicylaldimine nickel (II) is reported. Their magnetic and spectral properties are correlated with those of solutions of the originally diamagnetic complex, and it is concluded that in both of these species and also in some of the solutions association of the complex occurs. On this basis a general explanation of the properties of bis-N-alkyl-salicylaldimine nickel (II) complexes is given.

INTRODUCTION

The ability of certain nickel (II) – salicylaldimine complexes, which are diamagnetic as solids, to become paramagnetic when dissolved in organic solvents has been examined by many workers (1–4). As indicated in the first paper of this series (5), several different explanations have been proposed. The earliest suggestion (1) was that in non-co-ordinating solvents such as chloroform, a temperature-dependent, partial conversion from a square planar to a tetrahedral configuration occurred. Later views required either the formation of distorted octahedral species as a result of solvent interaction, or a sufficient modification of the crystal field to give essentially a paramagnetic square planar form.

Recent work has ruled out any possibility that the complexes assume tetrahedral configurations. Dielectric polarization measurements (6) give values of the dipole moments which are too low for tetrahedral forms, and also the spectra of the salicylaldimine complexes (1–5, 7) show no similarities to those of known tetrahedral nickel (II) complexes (8, 9). That paramagnetism may arise from solvent interaction with the square planar complexes to give a distorted octahedral configuration has been concluded by Maki (10) on the basis of detailed quantum mechanical calculations. With a suitable choice of parameters, this approach appears to explain satisfactorily the spectra both of the solid complexes and of their solutions. However, Maki's interpretation of the solution paramagnetism is really based on calculations of the distribution of the planar molecules between singlet and triplet states, between which a small, temperature-dependent energy separation is produced by an axial perturbation of the molecule. Such a situation may result from any suitable axial perturbation, of which solvent interaction is just one possibility. Moreover, if solvent interaction occurred in all solvents, one might expect there to be some correlation between the base strength of the solvent and the degree of occupation of the triplet state. Magnetic measurements of a series of bis-N-alkyl-salicyl-aldimine nickel (II) complexes in various solvents (4) show no relation between base strength of the solvent and the degree of paramagnetism. Also, bis-N-methyl-salicyl-aldimine nickel (II) can be converted to a buff-colored, paramagnetic form on heating to 180° (11, 12) in the complete absence of solvent. Although the very low solubility of this form, as well as its color, suggest that it is not the species responsible for solution paramagnetism, it is clear that solvent interaction is not necessary to produce paramagnetism. The low solubility has been taken (12) as an indication that this species is an associated form of the planar complex, association possibly occurring through Ni—O intermolecular links.

Ballhausen and Liehr (13) have also examined the problem theoretically and have concluded that paramagnetism may result from a decrease in the effective crystalline field seen by the complexes on melting, or on dissolution in "non-complexing" solvents.

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Contribution from the Chemistry Department, University of British Columbia, Vancouver 8, B.C.

Such a decrease may well lower the splitting between the low-lying singlet and triplet states so that $h\nu$ is comparable with thermal energies. In this event the complex will show paramagnetism, and these authors presented an equation which would describe such a system. This is a general equation of state describing the thermal distribution of electrons between two energy levels. Although the application of this equation to the results of some magnetic measurements (14) leads to reasonable values of the energy separation between the two levels, this does not "prove" the equation nor is it possible to draw any conclusions from these measurements as to the true state of co-ordination of the nickel atoms in this situation.

There is general agreement, then, that the partial paramagnetism of the nickel (II) – salicylaldimine complexes arises from a distribution between singlet and triplet states, but there is still no agreement as to the geometry of the paramagnetic species. The occurrence of paramagnetism in the absence of a solvent is attributed to association giving an octahedral configuration about the Ni (II) atoms. Ferguson (15) has very recently found that spectroscopic and molecular weight studies of benzene and chloroform solutions of bis-N-methyl-salicylaldimine nickel (II) show that associated forms are present at the concentrations at which partial paramagnetism is observed. Hence the axial perturbation of the planar complex, causing a thermal distribution between singlet and triplet states, may be due to association of the molecules in "non-complexing" solvents, rather than to direct solvent interaction. With this in mind, it seemed worth while to attempt to isolate paramagnetic species from solutions of these complexes, and also to investigate the formation of paramagnetic species by heating of the solid complexes.

EXPERIMENTAL

Of 14 complexes prepared and studied, only three showed interesting features. They were bis-N-methyl-salicylaldimine nickel (II) and its 5-chloro and 5-bromo analogues; their preparation has been described previously (5).

The species of bis-N-methyl-5-chloro-salicylaldimine nickel (II) obtained were as follows.

(i) On heating the dark green diamagnetic complex (A) at 165° for 30 minutes, conversion to a light green form (B) occurred. This was very soluble in organic solvents, and paramagnetic. Values of the molar susceptibility at temperatures in the range 80° to 298° K are listed in Table I, and the resultant plot of $1/\chi_M$ versus T_1 gives a value of

TABLE I

Corrected molar susceptibilities $\times 10^6$ c.g.s. units

Temperature (°K)		110	177	231	298
Species (B)		19080	8950	5965	4606
Temperature (°K)	80	118	157	221	298
Species (C)	20370	12910	9489	6606	4909
Temperature (°K)	81	104	151	208	298
Species (G)	18650	13550	8905	6389	4494

NOTE: Species (G) = buff-colored form of bis-N-methyl-salicylaldimine nickel (II).

+72° for the molecular field constant, whence the effective magnetic moment at 25° is 2.90 Bohr magnetons. That this product has the same composition as the original compound was shown by analysis. (Found: Ni, 14.46; N, 6.89; C, 48.51; H, 3.41. Calc. for $NiC_{16}H_{14}O_2N_2Cl_2$: Ni, 14.83; N, 7.07; C, 48.51; H, 3.56%.)

(ii) Exposure to temperatures of 200–205° for 4 hours, or considerably longer periods at 170°, converted bis-N-methyl-5-chloro-salicylaldimine nickel (II) into a buff-colored

solid (C) almost insoluble in all common organic solvents. The values of molar magnetic susceptibilities over a temperature range are also shown in Table I, from which the molecular field constant is found to be $+10°$ and $\mu_{(eff)}$ (25°) = 3.33 Bohr magnetons.

Analysis showed it to have the same empirical formula as both the original compound (A) and the light green form (B). (Found: Ni, 14.22; N, 7.17. Calc. for Ni $C_{16}H_{14}O_2N_2Cl_2$: Ni, 14.83; N, 7.07%.)

(iii) Since earlier magnetic measurements (2, 3) have shown that the paramagnetism of this type of complex in chloroform solution approaches the normal value for two unpaired electrons as the temperature is lowered towards −78°, such solutions provide an alternative means of isolating paramagnetic species. Repeated partial freezing of chloroform solutions of bis-N-methyl-5-chloro-salicylaldimine nickel (II) in solid CO_2–ethanol mixtures, followed by decantation of the concentrated solution from the solid chloroform, eventually caused precipitation of a light green species (D), quite different in properties from the original form (A). The same product was obtained by the removal of the solvent under vacuum from a cooled (−46° to −64°) chloroform solution, or by precipitation from a cooled chloroform solution by the addition of ligroin. Each of these procedures gave only small amounts of species (D) which in all instances contained a small amount of the original diamagnetic complex (A). It was therefore not possible to make accurate magnetic measurements, although a measurement at 25° gave a gram susceptibility of 8.3×10^{-6} c.g.s. and therefore an approximate effective magnetic moment of 2.8 Bohr magnetons. Species (D) was not a solvate since there was a negligible weight loss on heating at 100° and conversion to the diamagnetic form (A) occurred.

The positions of the absorption bands in the diffuse reflectance spectra of these various species are listed in Table II together with the bands observed for solutions of bis-N-methyl-salicylaldimine nickel (II) in chloroform and pyridine (10). The diffuse reflectance spectra were kindly determined by Dr. M. C. R. Symons by a procedure previously described (16).

TABLE II

Species	Observed maxima(mμ)			
A	440	620	—	—
B	480 (sh)	630	820 (sh)	ca. 1000
C	420 (sh)	560	780 (sh)	900
D	430 (sh)	610	780 (sh)	ca. 930
A* in $CHCl_3$	490 (sh)	610	(800 (sh)?)	1150
A* in C_5H_5N		570	785 (sh)	960

NOTE: A* = bis-N-methyl-salicylaldimine nickel (II); sh = shoulder.

The X-ray diffraction powder photographs of the various solid species were obtained with a G. E. model XRP-SF11 X-ray diffraction unit. The photographs of species (B) and (D) were very similar in line spacings and relative intensities, although not completely identical. Both were quite different from that of either species (A) or (C). Some slight differences might be expected in the crystal packings of (B) and (D) in view of the very different conditions under which they were obtained.

Previous reports (11, 12) of the effect of heat on bis-N-methyl-salicylaldimine nickel (II) are in disagreement. Harris, Lenzer, and Martin (12) obtained a buff-colored compound, while Sacconi *et al.* (11) reported the isolation of a light green product. An examination of the magnetic measurements of Sacconi's product shows that their original sample was slightly paramagnetic, presumably due to traces of bis-salicylaldehyde nickel (II), which would certainly give the final product a green color. We have found that on

heating a sample of the diamagnetic complex ($\chi_g = -0.307 \times 10^{-6}$ c.g.s.) to 200° for 2 hours conversion to a paramagnetic, buff-colored product occurred. There was no sign of the formation of any green paramagnetic product in the temperature range 150–210° and our results entirely confirm those of Harris *et al.* Measurements of the magnetic susceptibility are indicated in Table I, and these give a value of 10° for the molecular field constant and hence μ_{eff} (25°) is 3.23 Bohr magnetons in good agreement with the results of Harris *et al.*

Bis-N-methyl-5-bromo-salicylaldimine nickel (II) was converted to a light green form (at 175°) and then to a buff-colored form (at 220°). For both this complex and bis-N-methyl-salicylaldimine nickel (II), light green products were obtained from their chloroform solutions at low temperatures but no detailed studies of these were made. The heating of 14 other nickel (II) – salicylaldimine complexes to 350° for several hours did not cause conversion to other forms, although in certain cases decomposition occurred.

Analyses of nickel were made gravimetrically by precipitation with dimethylglyoxime. Nitrogen was determined by the standard semimicro Kjeldahl method.

Magnetic susceptibilities were measured with a Gouy magnetic balance fitted with a cryostat similar to that described by Figgis and Nyholm (17). The magnetic balance consisted of a Varian 4-in. electromagnet, model V4084, with 2-in. tapered pole caps, in conjunction with a Spoerhaese Model 10 M microbalance with a sensitivity of ±0.01 mg. Current regulation was provided by a Varian Model U 2300 A power supply and Model 2301 A current regulator. The cryostat used for temperature control of the sample is shown in Fig. 1. The general principles of design and the associated electrical circuits

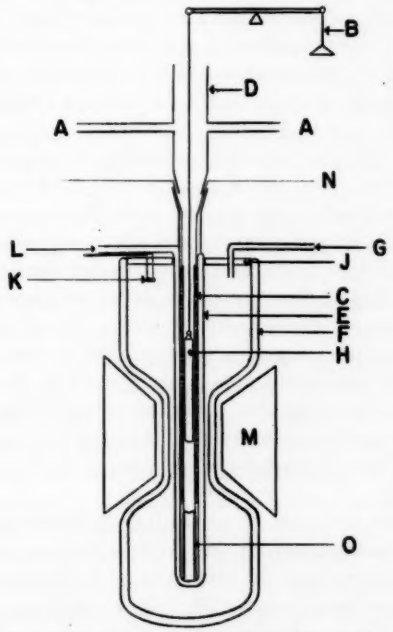

FIG. 1. Cryostat for the magnetic balance. A, nitrogen gas inlet and outlet; B, balance; C, heater and platinum resistance thermometer wound on brass former; D, draught shield; E, inner Dewar; F, outer Dewar; G, liquid nitrogen inlet; H, sample tube; J, icing shield; K, thermistor; L, to the pump for evacuation of the inner Dewar; M, magnet pole caps; N, connection to the heater and resistance thermometer; O, support for heater former.

of the cryostat are the same as described by Figgis and Nyholm. With this apparatus, temperatures can be maintained constant at any point in the range 78°–300° K by suitable control of the current passing through the electrical heating coil and of the pressure within the inner Dewar. A device is provided for maintaining constant the level of liquid nitrogen in the outer Dewar. The main modification of Figgis and Nyholm's design is in the shape of the outer Dewar. In our apparatus, the outside diameter of the outer Dewar is only 2.6 cm between the pole caps, compared with 7 cm. This makes it much simpler to obtain a reasonably large homogeneous region of the field and still use tapered pole caps and a shorter sample. Moreover the temperature control is perhaps improved by having a reservoir of refrigerant both above and below the constriction. The sample tube and apparatus were calibrated using mercury (II) cobaltitetrathiocyanate (18) as standard. Magnetic moments were calculated using the formula $\mu = 2.839 \sqrt{\{\chi_M \times (T+\theta)\}}$ Bohr magnetons.

DISCUSSION

The above results indicate the ability of bis-N-methyl-5-chloro-salicylaldimine nickel (II), and its 5-bromo analogue, to become paramagnetic under four sets of conditions, namely (i) heating to 165° to give the light green species (B), (ii) heating to 200–205° to form the buff-colored product (C), (iii) dissolution of the diamagnetic complex in organic solvents, and (iv) separation as a light green species (D) from such solutions in organic solvents. The experimental results show that there is considerable similarity between species (B) and (D). Their absorption spectra are very similar, both in the positions of the bands and in their relative intensities. Their X-ray diffraction powder photographs are also very similar although not quite identical. It is then reasonable to conclude that forms (B) and (D) are identical and also that they are probably identical with the paramagnetic species present in the chloroform solution from which form (D) was obtained. Species (C), the buff-colored product, is quite different from the other forms, as shown by its color, its very low solubility in organic solvents, and its very different diffraction pattern. For bis-N-methyl-salicylaldimine nickel (II), only the buff-colored form was obtained on heating the solid diamagnetic complex, and it seems that quite minor differences in the properties of the complexes determine whether conversion to this buff form will take place directly or through the light green form. In general, it seems a reasonable conclusion that these complexes are capable of existing as diamagnetic species, as green, paramagnetic soluble forms, or as buff-colored paramagnetic insoluble forms. Since both paramagnetic species exist in the absence of solvent, they presumably have distorted octahedral configurations about the nickel atoms as a result of association of the complex molecules. This has already been suggested for the buff form of bis-N-methyl-salicylaldimine nickel (II) (12) and the results of Ferguson, indicating association of the complex in chloroform solution, make it probable also for the green paramagnetic species.

Satisfactory explanations can now be made for all observations of these anomalous complexes. Clearly the paramagnetism of their solutions in strongly co-ordinating solvents such as pyridine results from the formation of octahedral, presumably monomeric species, some of which have been isolated (3, 19). In solvents which are normally considered non-co-ordinating (e.g. chloroform, cyclohexane, carbon tetrachloride), the removal of the crystalline forces which have restrained the molecule in its diamagnetic form, allows association of the complex and a decrease of the energy separation between

singlet and triplet states. Thus, an axial perturbation does occur, not through solvent interaction but rather by association. The same effect occurs on heating the solid when some breakdown of the crystal structure leads to the formation of the same associated green product. These conclusions are consistent with the dielectric polarization measurements (6), with the spectra of the complexes and their solutions (1, 15, 20), and with the magnetic measurements (3, 6).

Sacconi *et al.* (14), from magnetic and viscosity measurements, conclude that there is no evidence for the association of the nickel (II) – N-alkyl-salicylaldimine complexes. They report a practically linear relationship, for bis-N-octyl- and bis-N-nonyl-salicylaldimine nickel (II) in the molten state, between ln η and $1/T$ over a wide temperature range. However, examination of their results shows that while there is certainly linearity for temperatures above about 120° C, below this temperature there are small but definite departures from linearity. Moreover, magnetic measurements of the N-octyl complex in dibutyl phthalate solution (14) show, in a plot of μ versus T, a minimum in μ which is shifted towards higher temperatures and approaches 120° at higher concentration. Both this concentration effect and the non-linearity of viscosity can be explained in terms of association of the complex. It is interesting to note that magnetic measurements (21) of the melts of many of these N-alkyl-salicylaldimine complexes give a minimum value of the magnetic moment at approximately 120° in the plots of μ versus T.

Different effects therefore seem to be occurring below and above 120°. At temperatures below and up to approximately 120°, we conclude that there is a distribution between singlet and triplet states, the small energy separation between these two states being a consequence of association. The degree of association will be concentration dependent and also temperature dependent, so that as the temperature increases towards 120°, the population of the triplet state will decrease. Above approximately 120°, it is possible that the energy separation between the two energy levels may remain practically constant, as suggested by Sacconi *et al.* (14) so that an increase in thermal energy may increase the population of the lowest triplet state. Alternatively the observed increase in magnetic moments (14) for temperatures higher than 120° may be due to conversion to the insoluble buff-colored species. However, this conversion has only been observed for the N-methyl compounds and not for the higher members of the alkyl series.

The question of the manner of association of the complexes cannot be settled unambiguously without complete structure determinations of both the buff-colored and light green paramagnetic species of bis-N-methyl-5-chloro-salicylaldimine nickel (II). The magnetic moments unfortunately do not allow a distinction to be made between tetrahedral and octahedral configurations since although tetrahedral nickel complexes containing four identical ligands have moments of 3.5 Bohr magnetons or higher (9), tetrahedral complexes with several different ligands about the nickel atom are known to have moments of about 3.0 Bohr magnetons (22). However, the large values of the molecular field constant, at least in one case, can be explained as due to distortion from a regular octahedral arrangement and also by the fact that the splitting between the lower triplet and next singlet state is not large. It has already been suggested (12) that the buff-colored species of bis-N-methyl-salicylaldimine nickel (II) is polymeric with a six-covalent arrangement about each nickel atom due to interaction of each such atom with oxygen atoms of the molecules above and below it. If this be accepted, then the associated light green paramagnetic forms may involve interaction between the nitrogen atoms of one molecule with the nickel atoms of other molecules to give an octahedral arrangement. It is also interesting to notice that Ferguson (15) has recently prepare and orthogonal

form of bis-N-methyl-salicylaldimine nickel (II) in which the Ni—Ni distance is 3.30 Å, compared with 5.32 Å in the monoclinic form, emphasizing the possibility of quite close packing of the molecules in associated forms.

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ARYLATION OF QUINONES BY DIAZONIUM SALTS

VII. SYNTHESIS AND STRUCTURE OF SOME ARYL-CHLORO-*p*-BENZOQUINONES[1]

JEHANBUX F. BAGLI[2] AND PH. L'ÉCUYER

ABSTRACT

A careful re-examination of the arylation (P. Brassard and P. L'Écuyer. Can. J. Chem. **36**, 814 (1958)) reaction with chlorobenzoquinone[3] has revealed in its products the presence of all the three possible disubstituted isomeric quinones. Arylation using diazotized *p*-nitroaniline has resulted in the isolation of the three new isomeric quinones, whose structures are assigned, based on chemical and spectral evidences. Mechanistic implications in the arylation and halogenation of benzoquinones are discussed.

The Meerwein reaction (2) provides a general method of arylation of activated double bonds, using aryl diazonium salts, in the presence of copper salts as catalysts. Arylation of benzoquinone by diazotized aniline using typical Meerwein conditions (80% acetone–water solvent system, cupric chloride catalyst, and acetate buffer) is found to give crude phenylbenzoquinone in poor yield (<10% of crude product). Modified Meerwein conditions[4] (variation of the solvent system or the type of buffer) have been used for the arylation of benzoquinones. It has been recently (3) demonstrated that excellent yields of monoaryl benzoquinones are obtained by conducting the reaction in a completely aqueous medium, in the presence of an acetate buffer, and without the copper catalyst. An application of the procedure to the arylation of the monosubstituted benzoquinones has also been described (1, 4). However, the latter reactions are complicated by the fact that theoretically three isomers can be formed. The substituent present in the starting quinone and the stability of the diazonium salt are the major factors responsible for influencing the yields of these isomers. The arylation of toluquinone and phenylbenzoquinone with $ArN_2^+Cl^-$ (Ar = *p*-chlorophenyl) yields a single isolable disubstituted benzoquinone in reasonable yield (86% and 43% respectively) as against that of chlorobenzoquinone (1) which gives two isomeric chloro-*p*-chlorophenylbenzoquinones (III*a* and III*c*). The last mentioned result strongly suggests the possibility of the formation of all three isomers in the course of this reaction.

The investigation of the arylation of chlorobenzoquinone was therefore resumed with a view to the preparation of these isomers using benzene, *p*-chlorobenzene, and *p*-nitrobenzene diazonium chlorides as the arylating agents.

In the previous studies (1, 4) and in synthetic studies with benzoquinones in general little or no attempt has been made to use chromatography as a means of separation of the products. The use of ordinary alumina is impeded by the ease with which a molecule of benzoquinone can accept two electrons from a nucleophile to go over to the dianion of the corresponding hydroquinone. We found that such a conversion on acid alumina is slow but nevertheless unavoidable. Silica gel has proved to be a satisfactory adsorbent for the separation of substituted benzoquinones with complete inhibition of reduction on the column. It is of interest to note that the order of elution of the chloro-aryl-

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[2]*National Research Council postdoctoral fellow 1959–60.*
[3]*The term "benzoquinone" throughout this publication refers to p-benzoquinone.*
[4]*For earlier references see reference 3.*

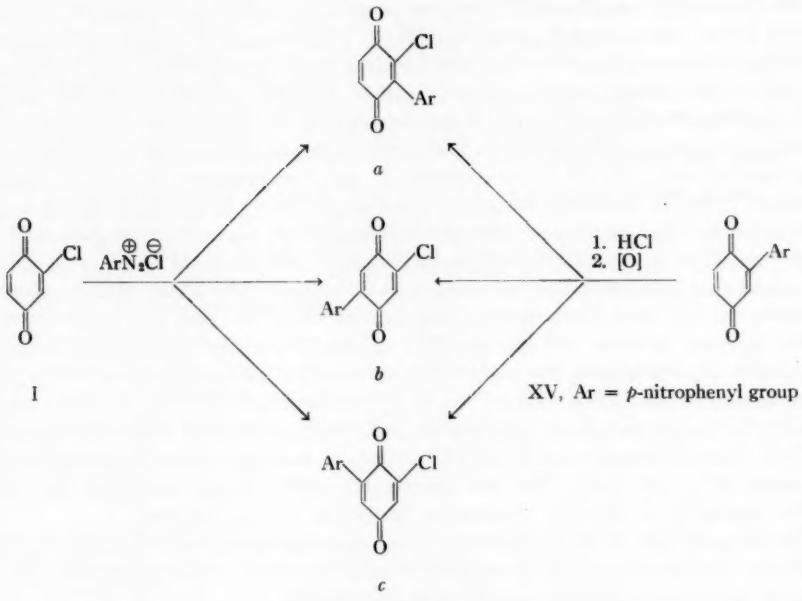
benzoquinones from a column bears a definite relationship to their structure. In all the cases studied, the degree of adsorbability of the disubstituted isomers follows the order: 2,3 > 2,6 > 2,5.

RESULTS AND DISCUSSION

Arylation of chlorobenzoquinone (I) (Chart I) with diazotized aniline gives the three isomers: 2-chloro-3-phenylbenzoquinone (II*a*), 2-chloro-5-phenylbenzoquinone (II*b*), and 2-chloro-6-phenylbenzoquinone (II*c*), in contrast to one isomer (II*b*) reported before (1).

CHART I

Arylation of chloroquinone and chlorination–oxidation of quinone XV



II, III, or IV

II, Ar = phenyl group

III, Ar = *p*-chlorophenyl group

IV, Ar = *p*-nitrophenyl group

Their structures are proven by their unequivocal synthesis (5). From the reaction of *p*-chlorobenzene diazonium chloride with I, besides 2-chloro-3-*p*-chlorophenylbenzoquinone (III*a*) and 2-chloro-6-*p*-chlorophenylbenzoquinone (III*c*) already described (1), the third isomer, 2-chloro-5-*p*-chlorophenylbenzoquinone (III*b*) is also isolated. The structures of all the three isomers of this series are confirmed by their infrared spectra (6).

The arylation of I with diazotized *p*-nitroaniline yields three disubstituted benzoquinones: 2-chloro-3-*p*-nitrophenylbenzoquinone (IV*a*), 2-chloro-5-*p*-nitrophenylbenzoquinone (IV*b*), and 2-chloro-6-*p*-nitrophenylbenzoquinone (IV*c*). The same three compounds are also obtained from the chlorination of *p*-nitrophenylbenzoquinone (XV) with concentrated hydrochloric acid, followed by oxidation with ferric chloride. The ratio of the isomers IV*a*, IV*b*, IV*c* obtained from the arylation of chloroquinone is 2:1:1; whereas that of the isomers secured from the chlorination–oxidation of *p*-nitrophenylquinone is about 0.1:2:1. Variation of the conditions for chlorination, e.g. passing dry HCl gas in a benzene or chloroform solution of the quinone XV, gave essentially the same results. The structures of these three isomers are assigned on the basis of their infrared (6) and nuclear magnetic resonance spectra. The N.M.R. spectrum of the compound IV*a* showed a nine-cycle spin coupling between the two adjacent quinoid hydrogen atoms whereas that of compound IV*c* showed a relatively weaker coupling of 2.5 cycles. Benzoquinone IV*b*, on the other hand, does not exhibit spin coupling between the two adjacent quinoid hydrogen atoms.

The above structural assignments are further substantiated by chemical evidence, obtained from the results of chlorination–oxidation experiments with each of these isomers (Chart II). Compound IV*c* on treatment with HCl followed by oxidation yields 2,3-dichloro-6-*p*-nitrophenylbenzoquinone (X) and 2,5-dichloro-3-*p*-nitrophenylbenzoquinone (VIII) in the ratio 1:5. Isomers IV*a* and IV*b* under identical treatment give respectively as a predominant product quinones VIII and X, together with traces of 2,6-dichloro-3-*p*-nitrophenylbenzoquinone (IX) as evidenced by infrared spectra (*vide*: Experimental). Trichloro-5-*p*-nitrophenylbenzoquinone is also isolated in small amounts (10–20%) from the chlorination–oxidation experiments with IV*a*, IV*b*, and IV*c*. Authentic samples of VIII, IX, and X are prepared by coupling diazotized *p*-nitroaniline with the corresponding 2,5-dichlorobenzoquinone (V), 2,6-dichlorobenzoquinone (VI), and 2,3-dichlorobenzoquinone (VII).

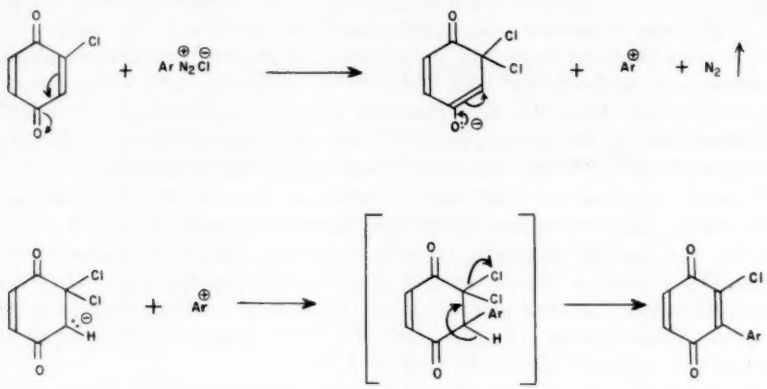
Treatment of hydroquinone with bromine as described (18), followed by oxidation with ferric chloride, results in the formation of 2,5-dibromobenzoquinone (XII), 2,3-dibromobenzoquinone (XIII), and tribromobenzoquinone (XIV), which are separated chromatographically.

The free radical nature of the Meerwein reaction is conclusively established by the recent work of Kochi (7). The oxidation–reduction capacity of the copper catalyst in presence of acetone is a unique feature of this mechanism. Absence of both the acetone and the copper catalyst makes such a mechanism highly improbable for the arylation under consideration. However, the problem of whether the arylation in the aqueous acidic medium proceeds via a free radical or an ionic pathway is still unresolved.

It is observed (from infrared spectra) that the decomposition of the diazonium salts into phenols and azo compounds according to their stability and the conversion of the quinones into quinhydrones (8) according to their redox potential compete with the main reaction.

It has been demonstrated by DeTar *et al.* (9) that the course of SN_1-type decomposition of the diazonium salts is largely governed by the pH of the mixture. The reaction follows a free radical pathway (10) under basic conditions, in contrast to an ionic pathway in an aqueous acidic medium. In view of these facts, a possible ionic mechanism for

the arylation may be formulated as follows:



Ar = aryl group

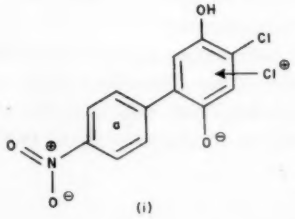
The experimental results from the chlorination–oxidation treatment of arylated chloroquinones IV*a*, IV*b*, and IV*c* are noteworthy.

In the predominant product X obtained from 2-chloro-5-*p*-nitrophenylbenzoquinone (IV*b*), the second chlorine atom enters ortho to the first one. On the other hand, in the formation of compound VIII from 2-chloro-6-*p*-nitrophenylbenzoquinone (IV*c*), the second chlorine atom enters ortho to the phenyl group. The substitution therefore seems to be independent of the bulk of the groups present. The generally accepted concept of 1,4 addition (11, 12) of HX (X = Cl or CN) to benzoquinone, by itself fails to explain the variation in the proportions of the isomers obtained from quinones XV, IV*a*, and IV*b*. This concept, however, assumes the prior chlorination of the quinone nucleus followed by aromatization to yield the chlorohydroquinones.

If one assumes that the aromatization of the quinone nucleus by an anion precedes the substitution as suggested by Clark (13) and more recently by Dewar (14), the subsequent substitution will then be governed by the principles of electrophilic aromatic substitution. The formation of compounds VIII and X as a major product from quinones IV*a* and IV*b*, now becomes readily explicable.[5]

The results of chlorination–oxidation of the quinone XV are, however, not explained by this latter view. Thus it seems reasonable that the addition of HX (X = Cl or CN) type reagents to benzoquinones does not involve either one or the other mechanism

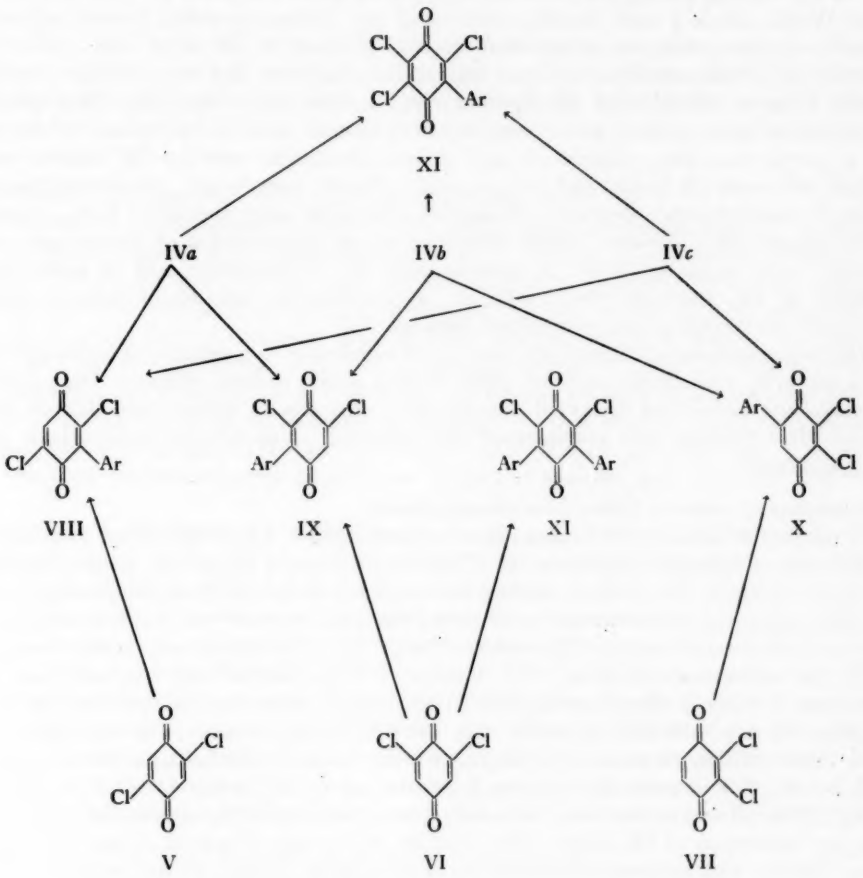
[5]*The prior aromatization can generate a transition complex of the type (i) from IV*b*, in which the nitro group can exert its electronic influence through the phenyl ring (a).*



(i)

CHART II

Chlorination–oxidation of quinones IV*a*, IV*b*, and IV*c* and arylation of dichloroquinones V, VI, and VII



exclusively. It is suggested that both pathways may be operative, depending upon the substituents present on the quinone nucleus and therefore upon the oxidation–reduction potential of the benzoquinone involved.

EXPERIMENTAL

General

All the melting points are uncorrected. Davison grade-923 silica gel (100–200 mesh) and Woelm grade-I acid alumina were used for chromatography. Unless otherwise mentioned, the term pet. ether refers to that fraction of petroleum ether with b.p. 30–60°. Ether extracts were all dried over sodium sulphate and ether always removed under vacuum. All infrared (IR) spectra were recorded with a Beckman IR-4 spectrophotometer using a NaCl prism; and reported spectra were all calibrated by the use of a polystyrene film. Chloroform and carbon disulphide used for IR spectra were ANALAR grade (B. D. H.) and reagent grade (Merck) respectively. Ultraviolet spectra were all recorded with a Beckman DU spectrophotometer using hexane (B. D. H., spectroscopic grade) as a solvent. Unless otherwise stated, diazotization of amines was conducted using amine (1 mole), in approximately 8% hydrochloric acid (3 moles) and adding, to the precooled (0–5°) solution, a concentrated solution of sodium nitrite (1 mole), maintaining the temperature between 0–5°.

Oxidation with ferric chloride was conducted as follows: to a solution of hydroquinone in acetic acid, was added a solution (60%, w/v in water) of ferric chloride. The reaction mixture was heated for about 10 minutes on a steam bath, cooled, and diluted with water. The quinone thus precipitated was collected, washed with water, dried, and recrystallized.

Preparation of Isomeric Chloro-phenylbenzoquinones

A solution of benzene diazonium chloride (from aniline, 4.7 g) was added to a stirred suspension of chlorobenzoquinone (I) (7.15 g) in a solution of sodium acetate (15.3 g) in water (650 ml). The reaction mixture was stirred overnight at room temperature. The oil thus separated was extracted with ether, the ether extract was washed and dried, and the ether was removed. The residue (13 g) was dissolved in pet. ether – benzene (1:1) and chromatographed on acid alumina (390 g). Elution with the same solvent gave first 2-chloro-5-phenylbenzoquinone (II*b*) (1.3 g), which crystallized from ethanol in platelets, m.p. 129–130° (reported m.p. 130–131.5° (15)). Its identity was confirmed by a mixed melting point and IR spectra. Further elution with benzene and a mixture of benzene–ether of gradually increasing polarity up to 50% ether gave an oily eluate (5.4 g). This oil was mixed with the residue from the mother liquor obtained from the first crystallization of II*b* above. The mixture (5.7 g) was dissolved in benzene – pet. ether (33:67) and rechromatographed on acid alumina (174 g). Initial fractions gave more of II*b* (0.61 g). Further elution with the same solvent followed by pure benzene gave unidentified oils (1.73 g). Elution with benzene–ether (98:2) gave an oil (0.82 g) which was rechromatographed on silica gel (65 g). Elution with benzene – pet. ether (1:1) gave a solid (0.42 g) which, after two crystallizations from ethanol, yielded 0.3 g of 2-chloro-6-phenylbenzoquinone (II*c*), m.p. 106–110°. Further purification from the same solvent provided an analytical sample, m.p. 112–113°, ν^{CHCl_3}: 919, 1659, and 1688 cm^{-1}; ν^{CS_2}: 916 (=C(H)—H deformation, on the quinone nucleus), 1656, and 1684 cm^{-1} (=C=O stretching). λ_{max}: 230 mμ (4.37), 300 mμ (3.87), 378 mμ (3.56). Calc. for $C_{12}H_7O_2Cl$ (218.63): C, 65.92; H, 3.21. Found: C, 66.10; H, 3.31. The proof of structure was provided by its unequivocal synthesis (5).

Further elution with the same solvent pair gave an eluate (0.29 g) which crystallized, when triturated with ether at dry-ice temperature. The solid (0.24 g) was dissolved in

benzene – pet. ether (1:3) and passed through silica gel (15 g). The eluate (0.2 g), crystallized from pet. ether (b.p. 65–110°), gave 2-chloro-3-phenylbenzoquinone (II*a*) (0.15 g), m.p. 115–116°. Two more crystallizations provided an analytical sample, m.p. 117–118°, ν^{CHCl_3}: 849, 1683 cm^{-1}; ν^{CS_2}: 844 (=C—H deformation, on quinone nucleus), 1681, 1669 cm^{-1} shoulder (=C=O stretching). λ_{max}: 250 mμ (4.19), 376 mμ (3.32). Calc. for $C_{12}H_7O_2Cl$ (218.63): C, 65.92; H, 3.21. Found: C, 65.95; H, 3.22. The structure of this compound was proved by mixed m.p. and infrared spectra of a sample synthesized by an unequivocal route (5).

Preparation of the Isomeric Chloro-p-chlorophenylbenzoquinones

A solution of *p*-chlorobenzene diazonium chloride (from *p*-chloroaniline, 6.4 g) was poured into a suspension of chlorobenzoquinone (I) (7.15 g) in a solution of sodium acetate (15.3 g) in water (675 ml). The reaction mixture was stirred overnight at room temperature. The oily solid which had then separated, was extracted with ether, the ether extract was washed and dried, and the ether was removed. The residue (13 g) was taken in benzene – pet. ether (1:3) and chromatographed on silica gel (420 g). The chromatogram was developed[6] over a period of 8 hours and eluted with the same solvent. Earlier fractions yielded an unidentified oil (0.43 g). Further elution gave 2-chloro-5-*p*-chlorophenylbenzoquinone (III*b*) as a crystalline solid (2.43 g), m.p. 135–140°. Crystallizations from acetone–ethanol afforded an analytical sample, m.p. 147–148°, ν^{CHCl_3}: 914, 1674 cm^{-1}; ν^{CS_2}: 910 (=C—H deformation, on quinone nucleus), 1674 cm^{-1} (=C=O stretching). λ_{max}: 239 mμ (4.34), 303 mμ (3.85), 380 mμ (3.51). Calc. for $C_{12}H_6O_2Cl_2$ (253.06): C, 56.96; H, 2.39; Cl, 28.02. Found: C, 56.98; H, 2.39; Cl, 27.98.

Further elution with solvents of increasing polarity up to benzene – pet. ether (2:3), yielded a crystalline solid (2.63 g). One crystallization from ethanol gave bright red needles of 2-chloro-6-*p*-chlorophenylbenzoquinone (III*c*) (1.73 g), m.p. 150–151°. A mixed melting point with an authentic sample (1) was undepressed and the I.R. spectra were superimposable. Elution with benzene – pet. ether (1:1) yielded an oily solid (4.0 g). Trituration of this solid with ether at dry-ice temperature followed by one crystallization from acetone – pet. ether provided golden yellow plates of 2-chloro-3-*p*-chlorophenylbenzoquinone (III*a*) (2.1 g), m.p. 104–105° (reported m.p. 103–104° (1)).

Preparation of the Isomeric Chloro-p-nitrophenylbenzoquinones

(a) By Arylation of Chlorobenzoquinone

p-Nitroaniline (3.45 g) was diazotized as described (16). The solution of *p*-nitrobenzene diazonium chloride was added to a stirred suspension of chlorobenzoquinone (I) (3.55 g) in a solution of sodium acetate (7.65 g) in water (325 ml) and the reaction mixture stirred overnight. The brown amorphous powder thus obtained was collected, washed thoroughly with water, and dried. This solid (5.3 g) was dissolved in benzene and chromatographed on silica gel (260 g). Eluates obtained were combined into three fractions based on I.R. analysis: I (1.14 g), II (1.70 g), and III (1.20 g).

Fraction II was dissolved in benzene and rechromatographed on silica gel (93 g). Initial eluates gave 2-chloro-6-*p*-nitrophenylbenzoquinone (IV*c*) (0.77 g) which crystallized in reddish-brown needles (0.31 g) from chloroform, m.p. 205–211°. An analytical sample recrystallized from acetone – pet. ether had m.p. 212–213°. ν^{CHCl_3}: (saturated

[6]*Preliminary development was found to be essential. Changing the solvent to the next higher polarity (benzene – pet. ether, 3:7) resulted in poorer separation.*

solution) 919 (=C—H deformation, on quinone nucleus), 1353, 1531 (N—O stretching), 1657, 1682 cm^{-1} (=C=O stretching). Calc. for $C_{12}H_6O_4NCl$ (263.63): C, 54.67; H, 2.24. Found: C, 54.63; H, 2.14.

The residue from the mother liquor of the chloroform crystallization above was pooled with fraction I. A first crystallization of their mixture from chloroform yielded 0.2 g more of IV*c*. The filtrate was evaporated to dryness and the residue, when crystallized from ethanol, yielded 2-chloro-5-*p*-nitrophenylbenzoquinone (0.47 g) (IV*b*), m.p. 129–132°. An analytical sample had m.p. 134–135°. ν^{CHCl_3}: 913 (=C—H deformation, on quinone nucleus), 1353, 1533 (N—O stretching), 1679 cm^{-1} (=C=O stretching). λ_{max}: 260 mμ (4.26), 360 mμ (3.26). Calc. for $C_{12}H_6O_4NCl$ (263.63): C, 54.67; H, 2.24; N, 5.31. Found: C, 54.99; H, 2.22; N, 5.53.

Further elution of fraction II with benzene gave a solid (0.72 g) which crystallized from acetone – pet. ether (b.p. 90–120°) in yellow rhombs (0.58 g), m.p. 147–155°. This was combined with 0.78 g of crystals, m.p. 146–157°, obtained from crystallization of fraction III, from the same solvent pair. The mixture was crystallized twice to yield 2-chloro-3-*p*-nitrophenylbenzoquinone (IV*a*) (1.0 g), m.p. 157–158°. An analytical sample melted at 160–161°. ν^{CHCl_3}: 845, 853 (=C—H deformation), 1354, 1533 (N—O stretching), 1686, inflection at 1671 cm^{-1} (=C=O stretching). λ_{max}: 256 mμ (4.33), 350 mμ (3.41). Calc. for $C_{12}H_6O_4NCl$ (263.63): C, 54.67; H, 2.24; N, 5.31. Found: C, 54.61; H, 2.34; N, 5.47.

(b) By Chlorination of p-Nitrophenylbenzoquinone (XV)

p-Nitrophenylbenzoquinone, m.p. 135–136°, (5 g) was refluxed with concentrated hydrochloric acid (500 ml) for 2 hours, cooled to room temperature, and the reaction product filtered. The precipitate was washed free of acid with water, dried, dissolved in acetic acid (78 ml), and oxidized with ferric chloride solution (50 ml), to give a mixture of quinones (5.3 g). The quinones were dissolved in benzene and chromatographed on silica gel (300 g). Initial fractions yielded a semisolid (0.43 g). This solid was crystallized three times from acetone–ethanol to give pale yellow crystals (0.18 g), m.p. 196.5–198° which proved to be identical with 2,5-dichloro-*p*-nitrophenylbenzoquinone (VIII) by a mixed melting point with an authentic sample and I.R. spectra. Further elution gave 2-chloro-5-*p*-nitrophenylbenzoquinone (IV*b*) which, after being purified through silica gel and crystallized from acetone–ethanol, gave crystals (1.0 g), m.p. 134–135°. Continued elution yielded a solid (0.74 g), m.p. 200–207°. Crystallization from acetone – pet. ether gave 2-chloro-6-*p*-nitrophenylbenzoquinone (IV*c*) (0.5 g), m.p. 208–211°. The residue (0.14 g) from the mother liquor of this crystallization was crystallized from acetone – pet. ether (b.p. 90–120°) and 2-chloro-3-*p*-nitrophenylbenzoquinone (IV*a*) (0.055 g), m.p. 153–156°, was isolated. The I.R. spectrum of this sample was essentially superimposable with that of the analytical sample of IV*a* obtained by method (*a*) above.

Preparation of 2,5-Dichloro-p-nitrophenylbenzoquinone (VIII)

To a stirred solution of 2,5-dichlorobenzoquinone, m.p. 160–161°, (1.77 g) in a mixture of acetic acid (130 ml), water (12 ml), and sodium acetate (3.06 g), was added a solution of diazotized (16) *p*-nitroaniline (1.38 g). The reaction mixture was stirred overnight and the temperature was then raised to 45° for 2.5 hours. The reaction mixture was cooled and filtered. The precipitate was thoroughly washed with water, dried, and

crystallized from acetone–ethanol. Pale yellow crystals (0.66 g), m.p. 196–198°, were isolated. The filtrate was then poured on ice while a solid (1.14 g) separated. This solid was crystallized from ethanol, dissolved in benzene, and passed through silica gel (25 g). The eluate was evaporated to dryness and the residue (0.28 g), m.p. 194–198°, was added to the first crop of 0.66 g of yellow crystals, m.p. 196–198°, and recrystallized from acetone–ethanol. Pure 2,5-dichloro-*p*-nitrophenylbenzoquinone melts at 197–198°.

ν^{CHCl_3}: 889 (=C—H deformation, on quinone nucleus), 1354, 1533 (N—O stretching), 1686 cm^{-1} (=C=O stretching). Calc. for $C_{12}H_5O_4NCl_2$ (298.08): C, 48.33; H, 1.69; N, 4.68. Found: C, 48.51; H, 1.87; N, 4.77.

Preparation of 2,6-Dichloro-p-nitrophenylbenzoquinone (IX)

To a stirred solution of sodium acetate (3.06 g) in acetic acid (80 ml) and water (100 ml) 2,6-dichlorobenzoquinone, m.p. 120–121° (1.77 g) and finally a solution of diazonium salt (16) obtained from *p*-nitroaniline (1.38 g) were added. The reaction mixture was stirred overnight and then the temperature raised to 50° for 45 minutes. After cooling, the precipitate was collected, washed with water, dried (1.63 g), dissolved in benzene, and chromatographed on silica gel (102 g). Initial fractions gave a solid (0.85 g), which was further purified by passing through silica gel. 2,6-Dichloro-*p*-nitrophenylbenzoquinone (0.65 g), m.p. 138–139°, thus obtained, crystallizes from acetone – pet. ether in pale yellow prisms, m.p. 140–141°. ν^{CHCl_3}: 890 (=C—H deformation, on quinone nucleus), 1353, 1533 (N—O stretching), 1663, 1698 cm^{-1} (=C=O stretching). Calc. for $C_{12}H_5O_4NCl_2$ (298.08): C, 48.33; H, 1.69; N, 4.68. Found: C, 48.19; H, 1.75; N, 4.66.

Further elution yielded a solid (0.44 g), which was crystallized twice from acetone – pet. ether to provide yellow crystals (0.22 g) of 2,6-dichloro-3,5-di-*p*-nitrophenylbenzoquinone (XI), m.p. 222–224°. An analytical sample had m.p. 229–230°. ν^{CHCl_3}: 1350, 1534 (N—O stretching), 1663, 1692 cm^{-1} (=C=O stretching). Calc. for $C_{18}H_8O_6N_2Cl_2$ (419.17): N, 6.68. Found: N, 6.67.

Preparation of 2,3-Dichloro-p-nitrophenylbenzoquinone (X)

2,3-Dichlorohydroquinone (16 g), prepared as described by Conant and Fieser (17), was dissolved in acetic acid (133 ml) and oxidized with ferric chloride solution (130 ml) into dichlorobenzoquinone (VII) (13.04 g), m.p. 100–101° (reported m.p. 100–101° (18)).

ν^{CHCl_3}: 840 (=C—H deformation), 1692 cm^{-1} (=C=O stretching).

To a stirred solution of 2,3-dichlorobenzoquinone (1.77 g) and sodium acetate (3.06 g) in acetic acid (100 ml) and water (63 ml) was added a solution of the diazonium salt (16) prepared from *p*-nitroaniline (1.38 g). The reaction mixture was stirred overnight and the temperature then raised to 50° for 45 minutes. After cooling, the mixture was filtered and the residue washed and dried. The solid (1.3 g) thus collected, was dissolved in pet. ether – benzene (3:7) and chromatographed on silica gel (104 g). Initial fractions yielded 2,3-dichloro-*p*-nitrophenylbenzoquinone (0.59 g), m.p. 162–164°, which after two crystallizations from acetone–ethanol melted at 164–165°. ν^{CHCl_3}: 906 (=C—H deformation, on quinone nucleus), 1353, 1533 (N—O stretching), 1688 cm^{-1} (=C=O stretching). Calc. for $C_{12}H_5O_4NCl_2$ (298.08): C, 48.33; H, 1.69; N, 4.68. Found: C, 48.20; H, 1.70; N, 4.67.

Chlorination of Isomeric Chloro-p-nitrophenylbenzoquinones

Chloro-*p*-nitrophenylbenzoquinones (IV*a*), m.p. 159–160°; (IV*b*), m.p. 134–135°; (IV*c*), m.p. 210–211°, were refluxed with concentrated hydrochloric acid (0.29 mole per mole of quinone) for 1.5 hours, cooled, diluted with water, and the reaction product extracted with ether. The ether extract was dried and the ether removed. The residue thus obtained was oxidized with ferric chloride solution into a mixture of quinones.

(*a*) 2-Chloro-3-*p*-nitrophenylbenzoquinone (IV*a*) (0.211 g) gave a crude mixture of quinones (0.17 g). This was dissolved in benzene – pet. ether (2:3) and chromatographed on silica gel (10 g). Eluate in the first three fractions gave a material (0.02 g) the I.R. spectrum of which showed no characteristic bands due to $=\overset{|}{C}-H$ out-of-plane deformation on the quinone nucleus. This observation, coupled with the fact that this material was present in small amount in the crude mixtures obtained from chlorination of all the three isomers, suggested it to be trichloro-*p*-nitrophenylbenzoquinone. This was further confirmed by chlorination of 2,5-dichloro-*p*-nitrophenylbenzoquinone (VIII) under the above conditions. The I.R. spectrum of the addition product was superimposable with that of the material obtained from the chromatogram. Further elution gave a quinone (0.11 g) which crystallized from acetone–ethanol, yielding crystals (0.08 g), m.p. 197–198°. A mixed melting point and I.R. spectra proved this to be 2,5-dichloro-*p*-nitrophenylbenzoquinone (VIII). The residue (0.02 g) from the mother liquor showed in its I.R. spectrum a band at 1056 cm^{-1} and a pronounced hump at 1664 and 1699 cm^{-1} on the carbonyl maxima at 1686 cm^{-1}. These are characteristic absorptions of 2,6-dichloro-*p*-nitrophenylbenzoquinone (IX). Further processing of this residue by one crystallization from acetone–ethanol gave another 0.012 g of 2,5-dichloro-*p*-nitrophenylbenzoquinone. The I.R. spectrum of the residue from the mother liquor of this last crystallization showed a marked increase in the intensity of the bands at 1056, 1664, and 1699 cm^{-1}, indicating strongly the presence of 2,6-dichloro-*p*-nitrophenylbenzoquinone (IX) in the crude product. Further elution gave 0.031 g of an oil which the I.R. spectrum (3560 cm^{-1}, O—H stretching) indicated it to be unchanged hydroquinone.

(*b*) 2-Chloro-6-*p*-nitrophenylbenzoquinone (IV*c*) (0.203 g), when treated with hydrochloric acid and oxidized as described previously, gave 0.205 g of a crude product. This was dissolved in benzene – pet. ether (1:1) and chromatographed carefully on silica gel (20 g). Fractions eluted were combined based on their I.R. spectra.

Fractions 1 to 17 (0.027 g) consisted exclusively of 2,5,6-trichloro-*p*-nitrophenylbenzoquinone. Fractions 18 to 23 (0.007 g) were found to be a mixture of this quinone and 2,3-dichloro-*p*-nitrophenylbenzoquinone (X) from the I.R. spectra of the intermediate fractions. From fractions 24 to 32 a solid was obtained which crystallized from acetone–ethanol to yield reddish brown needles of X (0.011 g), m.p. 160–163°, identified by a mixed melting point and the I.R. spectra. Fractions 33 to 45 (0.056 g) yielded in one crystallization a solid (m.p. 150–170°) suggested by its I.R. spectrum to be a mixture of X and 2,5-dichloro-*p*-nitrophenylbenzoquinone (VIII). Fractions 46 to 71 (0.076 g) were crystallized from acetone–ethanol to yield pale yellow needles (0.06 g) of a quinone, m.p. 192–194°, found to be VIII by mixed melting point and their I.R. spectra.

(*c*) 2-Chloro-5-*p*-nitrophenylbenzoquinone (IV*b*) (0.204 g), when treated with hydrochloric acid and ferric chloride as described above, yielded 0.206 g of crude quinones. This was dissolved in benzene – pet. ether (1:1) and carefully chromatographed on silica gel (20 g). Fractions eluted were pooled based on I.R. spectra.

Fractions 2 to 25 (0.049 g) consisted mainly of trichloro-*p*-nitrophenylbenzoquinone as identified by their I.R. spectra. Combination 26 to 65 (0.076 g) from the spectra of the intermediate fractions was found to contain 2,3-dichloro-*p*-nitrophenylbenzoquinone (X). It crystallized from acetone–ethanol in clusters of reddish-brown needles (0.07 g), m.p. 160–162°. A mixed melting point with an authentic sample and comparison of their I.R. spectra, confirmed its identity. An I.R. analysis of fractions 66 to 84 (0.048 g), eluted with pet. ether – benzene (3:7) showed the carbonyl band at 1688 cm^{-1} flanked by shoulders of varying intensities (increasing from 66 to 84) at 1663 and 1699 cm^{-1} and appearance of a band of increasing intensity in the same order at 890 cm^{-1}. These were strongly suggestive of the presence of 2,6-dichloro-*p*-nitrophenylbenzoquinone (IX) but crystallization of combination 66 to 84 from acetone–ethanol gave 0.02 g of 2,3-dichloro-*p*-nitrophenylbenzoquinone (X). An I.R. spectrum of the residue from the mother liquor showed marked increase in the intensity of the bands at 1663, 1699, and 890 cm^{-1}. However, an attempt to isolate IX either by chromatography or by crystallization proved fruitless.

Preparation of 2,5- and 2,3-Dibromobenzoquinones (XII and XIII)

Treatment of hydroquinone (11 g) with 2 moles of bromine as described (18), gave a mixture of dibromohydroquinones. This was oxidized with a ferric chloride solution (100 ml) to yield a mixture of quinones (26 g). A 5.1-g portion of this was crystallized from benzene – pet. ether to give 2,5-dibromobenzoquinone (XII) (2.7 g) which, after one crystallization from acetone–ethanol, melted at 189–190° (m.p. 191° (19)). The residue (2.13 g) from the mother liquor of the first crystallization was dissolved in benzene – pet. ether (1:1) and chromatographed on silica gel (126 g). Elution gave in the first two fractions a solid (0.27 g). After three crystallizations of this solid from acetone – pet. ether tribromobenzoquinone (XIV) (0.15 g) was obtained as pale yellow platelets, m.p. 152–153°, (m.p. 155° (19), 150° (20)). λ_{max}: 295 mμ (4.01), 375 mμ (2.75); reported (20) λ_{max}: 296 mμ (4.03), 375 mμ (2.76). ν^{CHCl_3}: 886 (=C—H deformation), 1674, 1696 cm^{-1} (=C=O stretching).

By further elutions more 2,5-dibromobenzoquinone (0.42 g) crystallized from acetone – pet. ether and finally 2,3-dibromobenzoquinone (XIII) was obtained. This quinone crystallized from pet. ether (b.p. 65–110°) in prismatic needles (0.5 g), m.p. 124–125°. ν^{CHCl_3}: 836 (=C—H deformation), 1681 cm^{-1} (=C=O stretching). λ_{max}: 256 mμ (4.28), 362 mμ (3.45). Calc. for $C_6H_2O_2Br_2$ (265.91): C, 27.10; H, 0.76; Br: 60.13. Found: C, 27.29; H, 0.77; Br, 60.54.

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THE EFFECT OF BETA-DECAY ON THE EXCHANGE PROPERTIES OF THE RARE EARTH–EDTA COMPLEX IONS[1]

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ABSTRACT

It is shown that the rare earth ion Yb^{3+} is very resistant towards ordinary thermal exchange when it is complexed with the chelating agent EDTA in aqueous solution. However, when the complexed rare earth atom, as the 1.8-h Yb-177, emits a beta-particle, the daughter atom Lu-177 escapes readily from the chelate structure. Nuclear recoil arising from the beta-particle emission is shown not to be the cause of the escape of the daughter atom. It is suggested that the observed lability of the daughter atom is a result of a high degree of chemical reactivity of the chelate ion arising from the sudden change in atomic number of the central metal ion of the chelate structure.

INTRODUCTION

The emission of nuclear radiation from an atom is always accompanied by a corresponding recoil of the residual atom. Enhanced chemical reactivity of the daughter atom, as a result of the nuclear event, is also frequently observed. This paper reports the results of a preliminary study of these so-called "hot-atom" effects for the lanthanide isotope Lu-177, in a particular chemical environment, when it is formed as a result of β^- decay of its 1.8-h Yb-177 parent. The decay sequence may be written as:

$$^{177}\mathrm{Yb} \xrightarrow[T_{1/2} = 1.8\ \mathrm{h}]{\beta^- = 1.3\ \mathrm{Mev}} {}^{177}\mathrm{Lu} \xrightarrow[T_{1/2} = 6.8\ \mathrm{d}]{\beta^-} {}^{177}\mathrm{Hf}_{\text{stable}}.$$

As is shown above, the daughter Lu-177 is also radioactive, a fact which greatly facilitates observations of its chemical behavior.

The particular system examined was the exchange reaction in aqueous solution between hydrated Yb^{3+} cations and $^{177}LuX^-$, where X stands for the tetrabasic chelating agent ethylenediaminetetraacetic acid (EDTA). The entity $^{177}LuX^-$ was formed in solution as a result of the β^- decay of $^{177}YbX^-$. This system was chosen because previous work from this laboratory had shown that these complex ions are remarkably stable, and in particular the thermal exchange reaction between the chelated lanthanide ion and the simple ion is very slow (1, 2). Any enhancement of reactivity of the chelate ion, as a result of β^- decay of its central lanthanide atom, was therefore expected to be readily observable.

The parent nuclide, Yb-177, was made by neutron irradiation of samples of pure ytterbium which were highly enriched in the isotope of mass 176. The use of this enriched material, rather than natural ytterbium (for which Yb-177 is not the predominant radionuclide (3)) permitted some simplification in the experimental methods and also aided in the interpretation of the results.

EXPERIMENTAL METHODS AND RESULTS

Radioactive ytterbium was produced by neutron irradiation of a known quantity of ytterbium (as an aqueous solution of the nitrate salt) for 1 hour in an empty fuel rod

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position of the NRX reactor. The nitrate solution was prepared from ytterbium oxide which had been mass-separated at Oak Ridge National Laboratories, and contained 97.5% Yb-176, and 1.81% Yb-174. It was initially free of lutetium. The principal activity formed was 1.8-h Yb-177, by neutron capture in the 97.5% abundant Yb-176. A minor activity, 4.2-d Yb-175, was also produced by neutron capture in Yb-174. It will be shown below that the presence of this Yb-175 activity permitted the correction for thermal exchange to be determined directly.

After the irradiation, sufficient Na_4EDTA solution was added to the irradiated solution to complex approximately two-thirds of the Yb^{3+} ions present. The remaining uncomplexed Yb^{3+} ions were quantitatively removed by absorption on a Dowex-50 cation resin column. This procedure ensured that all the radioactive atoms were initially complexed with EDTA and that no excess of EDTA was present. Non-radioactive Yb^{3+} ions were then added to the test solution, together with 0.01 *M* ammonium acetate, pH = 6.5. (The time at which these additions were made was recorded as "zero time".) The concentration of both ionic species YbX^- and Yb^{3+} was 2.5×10^{-4} *M*. The pH of 6.5 was chosen to minimize the normal thermal exchange between these two species, previous studies (1) having shown that the rate of the thermal exchange between M^{3+} and MX^- ions varies directly with the acidity of the solution.

The decay of the 1.8-h Yb-177, and the concurrent "hot-atom" or thermal exchange reactions in solution, were allowed to proceed for 4.6 hours. During this time, 81.2% of the Yb-177 atoms present had decayed to Lu-177. The free Yb^{3+} ions (and any $^{177}Lu^{3+}$ ions which had found their way into this so called free-ion fraction as a result of recoil or thermal exchange processes) were then quantitatively removed from the test solution by absorption onto a Dowex-50 resin column. The absorbed species were then eluted off the column with 0.4 *M* α-hydroxyisobutyric acid solution of pH 6.5. Sufficient time, approximately 24 hours, was then allowed for the residual Yb-177 to decay completely to Lu-177, and then the latter nuclide in the free-ion fraction was separated from 4.2-d Yb-175 by chromatographic elution from a Dowex-50 resin column; this method has been described by Choppin and Silva (4). The separated Yb-175 and Lu-177 were set aside for further measurements outlined below.

1. The Number of Yb-177 Atoms in the Test Solution at Zero Time

This quantity was determined by absolute 4π-β^- counting of an aliquot of the test solution, the radioactive decay of the Yb-177 being followed for 10 half-lives. A total of 11.37×10^{10} atoms of Yb-177 was present in solution as $^{177}YbX^-$ at zero time. Of this number, 81.2% or 9.23×10^{10} atoms had decayed to Lu-177 in the 4.6-hour period allowed.

2. Measurement of the Number of Lu-177 Atoms in the Free-ion Fraction

Lu-177 atoms in the free-ion fraction could have found their way there by one or more of the following routes:

(i) "Hot-atom" effects associated with the β^- decay of $^{177}YbX^-$.

(ii) Thermal exchange of Yb^{177}: $^{177}YbX^- + Yb^{3+} \rightarrow YbX^- + {}^{177}Yb^{3+}$, followed by β^- decay of the Yb-177 atoms so released.

(iii) Thermal exchange involving the reaction: $^{177}LuX^- + Yb^{3+} \rightarrow YbX^- + {}^{177}Lu^{3+}$.

(Lu-177 atoms in the form $^{177}LuX^-$ are present in significant and calculable amount at zero time as a result of β^- decay of Yb-177 during the irradiation in NRX, and also in the time between the end of the irradiation and the time at which the EDTA chelate was formed.) The sum of these three contributions was determined in the following

way. The Lu-177 activity isolated from the free-ion fraction was measured on a 100-channel γ-ray spectrometer which had been previously calibrated with an independently standardized Lu-177 source. A total of 5.85×10^{10} atoms of Lu-177 was found in the free-ion fraction. It is now necessary to distinguish between the "hot atom" and thermal exchange contribution to this quantity.

3. Extent of Thermal Exchange

Earlier studies (1) suggested that thermal exchange would not be a significant effect for the conditions used in the present work, and this was confirmed by measurements of the extent of transfer of Yb-175 from the chelate ion to the free-ion fraction. These measurements were made by comparing the areas under the photopeak characteristic of Yb-175 in the γ-ray spectra of an aliquot taken from the test solution and an aliquot of the Yb-175 part of the free-ion fraction referred to earlier. A value of 2.8% was obtained for the extent of thermal exchange, a result somewhat higher than would have been predicted from earlier studies (1) but not grossly inconsistent with these earlier results. This result can be used to estimate the contribution of process (ii) above (thermal transfer of Yb-177), to the appearance of Lu-177 in the free-ion fraction. Thus, the *average* number of Yb-177 atoms available for thermal exchange during the 4-hour period may be taken to a good approximation as one-half the original number present, i.e., $0.5\times11.37\times10^{10} = 5.68\times10^{10}$ atoms. Transfer of 2.8% of these represents 0.16×10^{10} atoms of Lu-177 contributed to the free-ion fraction.

A similar assessment can be made of the extent of thermal transfer of Lu-177 from the form $^{177}LuX^-$ to $^{177}Lu^{3+}$. Earlier studies (1, 2) showed that the rate of thermal exchange of the lanthanide elements in the form MX^- decreases slowly with increasing atomic number. The 2.8% transfer of Yb-175 therefore represents also an *upper* limit of the percentage of lutetium atoms present as $^{177}LuX^-$, which underwent normal thermal exchange during the experiment. The number of Lu-177 atoms present in this form at zero time was 7.44×10^{10} atoms. This number was readily calculated from the number of Yb-177 atoms present at zero time, together with the time of irradiation, and the time between the end of the irradiation and the formation of the EDTA chelate. The number of $^{177}LuX^-$ present at the end of the run was 10.8×10^{10}. An average value of 9.1×10^{10} may be used for estimating the extent of thermal transfer. A maximum of 2.8% of these 9.1×10^{10} atoms would be transferred to the free-ion fraction in 4.6 hours, i.e., 0.25×10^{10} atoms.

The pertinent numerical results obtained above are summarized in Table I. Comparison of items B and C in Table I shows clearly that the number of Lu-177 atoms found in

TABLE I

Source and distribution of Lu-177 atoms

	No. of atoms $\times10^{-10}$
A. Formed in solution from decay of $^{177}YbX^-$ in 4.6 hours	9.23
B. Found in free-ion fraction	5.85
C. No. expected in free-ion fraction from thermal exchanges:	
(i) $^{177}YbX^- + Yb^{3+} \rightarrow YbX^- + {}^{177}Yb^{3+}$, $^{177}Yb^{3+} \rightarrow Lu^{3+} + \beta^-$	0.16
(ii) $^{177}LuX^- + Yb^{3+} \rightarrow YbX^- + {}^{177}Lu^{3+}$	0.25
Total contribution to B from thermal exchange processes	0.41
D. No. transferred by "hot-atom" processes = (B–C)	5.44
Net fraction of A transferred by "hot-atom" processes = D/A =	0.590

the free-ion fraction was very much greater than can be accounted for by thermal exchange processes. The presence of these extra Lu-177 atoms in the free-ion fraction is ascribed to "hot-atom" processes associated with the β^- emission from Yb-177, when this nuclide is present in the chemical form $^{177}YbX^-$. A comparison of items D and A, shown in the last entry of Table I, shows that 59% of the Lu-177 atoms formed by β^- decay of $^{177}YbX^-$ escape from the chelate and turn up in solution in the cationic form $^{177}Lu^{3+}$.

The results described above appear to be quantitatively reproducible; a second essentially identical experiment gave a value of 56% for the percentage of the Lu-177 atoms escaping from the chelate ion as a result of β^- decay of $^{177}YbX^-$. This is in good agreement with the value of 59% listed in Table I.

DISCUSSION

The maximum energy of the β^- particle emitted by Yb-177 is 1.3 Mev. A free, isolated Lu-177 daughter atom then receives, as a result of nuclear recoil, a *maximum* of 9 ev of kinetic energy. This value is given by the expression

$$E_R = \frac{541\,E_\beta}{M} + \frac{536\,E_\beta^2}{M}.$$

E_R is the recoil energy in electron volts, E_β is the maximum energy of the beta particle in Mev, M is the mass of the recoiling atom.

However, it is well known that the average β^- energy is approximately 0.4 E_β, so that an isolated Lu-177 atom will receive on the average only 3.5 to 4.0 ev of recoil. (The relation between the average recoil energy given to an isolated atom and the maximum β^- energy of the disintegration has been discussed by several authors, e.g. Libby (5).) Moreover, in the present study, the daughter atom is in fact not a free, isolated atom, but is linked by at least four strong co-ordinate bonds to the chelating agent EDTA (2, 6). The recoil energy is therefore shared by a more massive configuration, with the result that the average kinetic energy of translation of the recoiling Lu-177 will be significantly less than 3–4 ev. On the other hand, 2–3 ev would be a reasonable estimate of the binding energy associated with each of the four co-ordinate bonds in the chelate. It appears highly unlikely therefore that nuclear recoil, as such, contributes appreciably to the observed escape of Lu-177 from the chelate environment.

A more likely cause for the observed escape is the abrupt change in atomic number of the central atom of the chelate. The loss of a negative charge from the nucleus produces a rearrangement of the electronic energy levels of the daughter atom, and thereby introduces a high degree of electronic excitation (see, for example, a recent review by Baulch and Duncan (7)). One may suppose therefore either that this excitation leads directly to dissociation, or alternatively that an excited species is formed. A necessary corollary of the latter alternative is that the excited species, during its lifetime, is very much more labile with respect to exchange than an ordinary lanthanide–EDTA chelate. Further speculations would appear unwarranted at present, in view of the limited amount of experimental evidence yet available. In this connection, a useful line for future investigation would be to discover whether or not changes in the chemical environment (pH, temperature, concentration of free lanthanide ions) have any effect on this "hot-atom" system.

ACKNOWLEDGMENTS

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SOME STABLE DIMERS OF SUBSTITUTED BENZYLCYANAMIDES[1]

D. L. Garmaise and A. Uchiyama

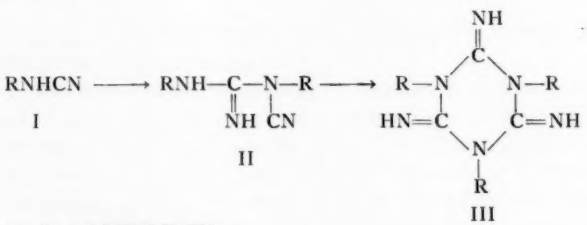
ABSTRACT

2,4- and 3,4-Dichlorobenzylcyanamides dimerize on standing to 1-cyano-1,3-di-(2,4-dichlorobenzyl)-guanidine and 1-cyano-1,3-di-(3,4-dichlorobenzyl)-guanidine respectively. Apart from dicyandiamide itself, no other cyanamide dimers appear to have been described. The dimers on heating are converted into the corresponding trisubstituted isomelamines. 1-Cyano-1,3-di-(3,4-dichlorobenzyl)-guanidine condenses with 4-nitrobenzylcyanamide to form 1,3-di-(3,4-dichlorobenzyl)-5-(4-nitrobenzyl)-isomelamine. No dimers were obtained from the 4-chloro-, 4-bromo-, 4-nitro-, or 4-methyl-benzylcyanamides.

Monosubstituted cyanamides trimerize slowly on standing, more rapidly when heated, and, in some cases, when subject to the action of strong alkali (1).

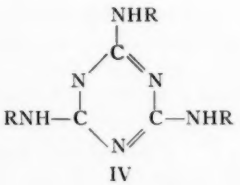
Alkylcyanamides (2, 3) and aralkylcyanamides (4) give trisubstituted isomelamines, while arylcyanamides may also yield the corresponding melamines (5).

No stable intermediate would be expected in the formation of melamines (IV), but the formation of isomelamines (III) should involve as intermediates, 1,3-disubstituted dicyandiamides (II).



I II III

a: R = 3,4-$Cl_2C_6H_3CH_2$—
b: R = 2,4-$Cl_2C_6H_3CH_2$—
c: R = 4-$ClC_6H_4CH_2$—
d: R = 4-$BrC_6H_4CH_2$—
e: R = 4-$O_2NC_6H_4CH_2$—
f: R = 4-$H_3CC_6H_4CH_2$—



IV

Derivatives of this type have not been previously reported.

It is now shown that when R = 3,4-dichlorobenzyl (*a*) or 2,4-dichlorobenzyl (*b*), the second step is sufficiently slow to permit the isolation and characterization of the dicyandiamides II*a* and II*b*.

Six substituted benzylcyanamides (Table I) were prepared by treating benzylamines with cyanogen bromide in ether (6). The products were readily isolated, generally in good yield, by operating at room temperature. The infrared spectra of the cyanamides showed the C≡N band at 2220–2240 cm^{-1} with no absorption in the C=N region except in the case of 3,4-dichlorobenzylcyanamide (I*a*). Even freshly prepared samples of I*a* showed a weak band at 1675 cm^{-1} in addition to the C≡N band.

[1]*Manuscript received January 31, 1961.*
Contribution No. 31 from the L. G. Ryan Research Laboratories of Monsanto Canada Limited, LaSalle, Quebec.

TABLE I

Benzylcyanamides RNHCN

R	Yield, %	M.p., °C	Formula	C		H		Hal.		N	
				Calc.	Found	Calc.	Found	Calc.	Found	Calc.	Found
4-Chlorobenzyl	76	91–92	$C_8H_7ClN_2$	57.68	57.51	4.23	4.36	21.28	21.64	16.81	16.37
4-Bromobenzyl	70	99–101	$C_8H_7BrN_2$	45.52	46.39	3.34	3.36	37.86	38.39	13.27	13.70
4-Nitrobenzyl	37	120–121	$C_8H_7N_3O_2$	54.24	54.41	3.98	4.16			27.32	23.62
4-Methylbenzyl	95	79–80	$C_9H_{10}N_2$	73.94	74.16	6.89	7.23			19.17	18.97
2,4-Dichlorobenzyl	94	60–61	$C_8H_6Cl_2N_2$	47.80	47.78	3.01	3.16	35.27	35.64	13.93	13.72
3,4-Dichlorobenzyl	93	48–50*									

*Not isolated free of the dimer.

Birkofer (4) reported that in attempting to purify benzylcyanamide by extraction with 30% sodium hydroxide solution in accordance with an earlier procedure (7), he observed an immediate precipitation of tribenzylisomelamine. We have found that 4-methylbenzylcyanamide (I*f*) is slowly converted to the isomelamine in 30% sodium hydroxide solution, but that benzylcyanamides with electron-withdrawing substituents (I*a*, I*b*, I*c*, I*d*) hydrolyze in this medium to the corresponding ureas instead of trimerizing. The 4-nitro analogue (I*e*) did not yield an identifiable derivative.

All of the cyanamides are converted to the isomelamines (III) when heated at 100°. The infrared spectra of the isomelamines show characteristically strong C=N bands at 1610–1620 cm^{-1}, with no adsorption in the C≡N region. I*c*, I*d*, and I*f* also formed the corresponding isomelamines on standing at room temperature for periods of 10–30 days, while the nitro analogue (I*e*) was unchanged in 30 days. The 2,4-dichloro analogue (I*b*), m.p. 60–61°, on being allowed to stand in an open vessel for 3 days, gave a 95% yield of a substance melting at 142–143°. This product (II*b*) showed strong bands at both 2245 cm^{-1} and 1690 cm^{-1}, indicating the presence of both C≡N and C=N bands. II*b*, on being melted and then heated at 100° for 4 hours was converted to the isomelamine III*b*, m.p. 220–222° in 75% yield.

Similarly, the 3,4-dichloro analogue (I*a*) (m.p. 48–50°), on standing in an open dish for 24 hours, gave a 63% yield of II*a*, m.p. 138–139°. II*a* also showed strong bands at 2240 cm^{-1} and 1674 cm^{-1}, and was converted in refluxing methanol to the isomelamine III*a*, m.p. 170–171° (8) in 70% yield. Paper chromatography in butanol:acetic acid:water, with development by bromcresol green gave R_f values of 0.73, 0.86, and 0.93 for I*a*, II*a*, and III*a* respectively.

Although reliable molecular weight determinations could not be made, the dimeric nature of II*a* was established by benzoylation to a monobenzoyl derivative, m.p. 200–201°. The benzoyl derivative analyzed for the formula $C_{23}H_{16}Cl_4N_4O$, which could be derived only from a dimer of the formula $C_{16}H_{12}Cl_4N_4$. The structure of the benzoyl derivative was not established.

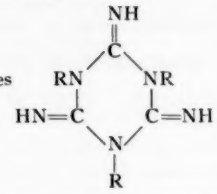
The dicyandiamide (II*a*) and 4-nitrobenzylcyanamide (I*e*) were allowed to stand in alcohol solution for 6 days, giving 1,3-di-(3,4-dichlorobenzyl)-5-(4-nitrobenzyl)-isomelamine in 20% yield. This result supports the assumption that 1,3-disubstituted dicyandiamides of the structure II are true intermediates in the trimerization of cyanamides to

isomelamines. 4-Nitrobenzylcyanamide was chosen for this reaction because of its relative stability at room temperature.

For purpose of comparison, a number of N^2,N^4,N^6-tribenzylmelamines (IV*b*, IV*c*, and IV*f*) were prepared by the reaction between cyanuric chloride and benzylamines. N^2,N^4,N^6-Tri-(3,4-dichlorobenzyl)-melamine has been previously described (8). The infrared spectra of the melamines showed C=N absorption at 1613–1624 cm^{-1}.

TABLE II

Isomelamines



R	Yield, %	M.p., °C	Formula	C Calc.	C Found	H Calc.	H Found	Hal. Calc.	Hal. Found	N Calc.	N Found
4-Chlorobenzyl	80	165–167	$C_{24}H_{21}Cl_3N_6$	57.68	58.01	4.23	4.42	21.28	21.31	16.81	16.53
4-Bromobenzyl	90	207–208	$C_{24}H_{21}Br_3N_6$	45.52	45.90	3.34	3.63	37.86	38.39	13.27	13.71
4-Nitrobenzyl	19	228–229	$C_{24}H_{21}N_9O_6$	54.24	54.34	3.98	3.94			23.72	23.62
4-Methylbenzyl	90	203–204	$C_{27}H_{30}N_6$	73.94	73.43	6.89	7.04			19.17	18.63
2,4-Dichlorobenzyl	65	220–222	$C_{24}H_{18}Cl_6N_6$	47.80	47.42	3.01	3.16	35.27	35.40	13.93	13.88
3,4-Dichlorobenzyl	70	171–172*									

*Literature reported m.p. 171–172° (8).

EXPERIMENTAL[2]

Preparation of Benzylcyanamides

Example.—Cyanogen bromide (7.55 g, 0.075 mole) in ether (100 ml) was added to a stirred solution of 4-chlorobenzylamine (21.4 g, 0.15 mole) in ether (100 ml) at 5° during 15 minutes. The suspension was stirred for 1 hour, and the precipitated 4-chlorobenzylamine hydrochloride was filtered. The ether solution was washed with 1 *N* hydrochloric acid (2×200 ml) and with water, and was then dried and evaporated. The crystalline residue of 4-chlorobenzylcyanamide (12.3 g), m.p. 67–70°, was recrystallized from ether to a constant melting point of 91–92°, yield 9.5 g (76% of theory).

Preparation of Isomelamines

4-Chlorobenzylcyanamide (0.50 g) was heated at 100° for 4 hours. Trituration with methanol gave 1,3,5-tri-(4-chlorobenzyl)-isomelamine, m.p. 162–165°, yield 0.40 g (80% of theory). Recrystallization from methanol raised the melting point to 165–166°.

In the case of the 4-nitro analogue (III*e*), the product had to be separated from relatively large amounts of colored by-products. The 4-chloro, 4-bromo, and 4-methyl analogues (III*c*, *d*, and *f*) could also be obtained by allowing the cyanamides to stand at room temperature for periods of 10–30 days.

[2]*All melting points are uncorrected. Analyses were performed by Micro-Tech Laboratories, Skokie, Ill., and Dr. Claude Daessle, Montreal, Quebec.*

Behavior of Benzylcyanamides in Aqueous Alkali

4-Methylbenzylcyanamide.—A solution of 4-methylbenzylcyanamide (1.5 g) in 30% sodium hydroxide (35 ml) was allowed to stand for 3 days. 1,3,5-Tri-(4-methylbenzyl)-isomelamine, m.p. 203–204°, slowly precipitated, yield 1.0 g (67%). There was no depression on admixture with a sample prepared as described above.

4-Chlorobenzylcyanamide.—4-Chlorobenzylcyanamide (0.50 g, 0.003 mole) was allowed to stand in 30% sodium hydroxide solution (20 ml) overnight. The precipitated 4-chlorobenzylurea, m.p. 185–186°, was filtered, yield 0.30 g (48%). Recrystallization from methanol raised the melting point to 189–190°. Acidification of the alkaline filtrate gave 0.11 g of unchanged 4-chlorobenzylcyanamide. Anal. Calc. for $C_8H_9ClN_2O$: C, 52.05; H, 4.91; Cl, 19.20; N, 15.18. Found: C, 51.96; H, 4.99; Cl, 19.08; N, 14.92.

3,4-Dichlorobenzylcyanamide.—Under the same conditions as described above, 3,4-dichlorobenzylcyanamide gave 3,4-dichlorobenzylurea, m.p. 163.5–164.5° (from methanol) in 77% yield. Anal. Calc. for $C_8H_8Cl_2N_2O$: C, 43.81; H, 3.68; N, 12.78. Found: C, 43.67; H, 3.58; N, 13.01.

4-Bromobenzylcyanamide.—Similarly, 4-bromobenzylcyanamide gave 4-bromobenzylurea, m.p. 201–203° (from ethanol), in 52% yield. Anal. Calc. for $C_8H_9BrN_2O$: Br, 34.89; N, 12.23. Found: Br, 34.75; N, 12.64.

Preparation of Melamines

Example.—Cyanuric chloride (3.7 g, 0.020 mole) was added to 4-methylbenzylamine (20.0 g, 0.165 mole) in *o*-xylene (100 ml) during 15 minutes, and the mixture was refluxed for 4 hours. The precipitated 4-methylbenzylamine hydrochloride was filtered, and the filtrate was evaporated to dryness. Crystallization of the residue from ethanol gave N^2,N^4,N^6-tri-(4-methylbenzyl)-melamine, m.p. 123–127°, yield 8.8 g (quantitative). The analytical sample melted at 129–130° after recrystallization from ethanol and from acetone. Anal. Calc. for $C_{27}H_{30}N_6$: C, 73.90; H, 6.91; N, 19.18. Found: C, 73.92; H, 7.05; N, 19.38.

N^2,N^4,N^6-Tri-(4-chlorobenzyl)-melamine.—N^2,N^4,N^6-Tri-(4-chlorobenzyl)-melamine, m.p. 158–159° (from ethanol) was obtained in 69% yield. Anal. Calc. for $C_{24}H_{21}Cl_3N_6$: C, 57.67; H, 4.24; Cl, 21.28; N, 16.81. Found: C, 57.65; H, 4.17; Cl, 21.27; N, 16.79.

N^2,N^4,N^6-Tri-(2,4-dichlorobenzyl)-melamine.—N^2,N^4,N^6-Tri-(2,4-dichlorobenzyl)-melamine, m.p. 155–157°, was isolated in 88% yield. Anal. Calc. for $C_{24}H_{18}Cl_6N_6$: C, 47.81; H, 3.01; Cl, 35.26; N, 13.95. Found: C, 47.64; H, 3.08; Cl, 35.26; N, 13.95.

1-Cyano-1,3-di-(2,4-dichlorobenzyl)-guanidine

2,4-Dichlorobenzylcyanamide (1.0 g, m.p. 60–61°) was allowed to stand in an open crystallizing dish for 2 days. Trituration with ether yielded the product melting at 140–143°. The ethereal mother liquors on standing for 24 hours deposited an additional quantity of the same substance, m.p. 140–142°. Total yield 0.95 g (95%). Recrystallization from methanol (30 ml/g) raised the melting point to 142–143°. Anal. Calc. for $C_{16}H_{12}Cl_4N_4$: C, 47.80; H, 3.01; Cl, 35.27; N, 13.93. Found: C, 47.35; H, 3.04; Cl, 35.37; N, 13.70.

The product (0.20 g) was heated above its melting point (150–160°) for 10 minutes and then heated at 100° for 4 hours. Trituration with methanol yielded crude 1,3,5-tri-(2,4-dichlorobenzyl)-isomelamine, m.p. 210–217°, yield 0.15 g (75%). Recrystallization from dimethylformamide–methanol–water raised the melting point to 220–222°. There was no depression on admixture with an authentic sample prepared by direct trimerization of 2,4-dichlorobenzylcyanamide.

1-Cyano-1,3-di-(3,4-dichlorobenzyl)-guanidine

3,4-Dichlorobenzylcyanamide (13.0 g, m.p. 48–50°) was allowed to stand in an open crystallizing dish for 24 hours. During this period the starting material melted and later partially resolidified. Trituration with ether yielded the product, m.p. 128–134°, yield 8.2 g (63%). The product was recrystallized to a constant melting point of 138–139° by dissolving in preheated methanol (30 ml/g) and cooling rapidly. Anal. Calc. for $C_{16}H_{12}Cl_4N_4$: C, 47.80; H, 3.01; Cl, 35.27; N, 13.93. Found: C, 47.57; H, 3.24; Cl, 35.58; N, 13.93.

A solution of 1-cyano-1,3-di-(3,4-dichlorobenzyl)-guanidine (0.50 g) in methanol (50 ml) was refluxed for 3 hours. The solution was evaporated and the residue was crystallized from ether, giving 1,3,5-tri-(3,4-dichlorobenzyl)-isomelamine, m.p. 167–169°, yield 0.35 g (70%). There was no depression on admixture with a sample, m.p. 171–172°, prepared by direct trimerization of 3,4-dichlorobenzylcyanamide.

Benzoylation of 1-Cyano-1,3-di-(3,4-dichlorobenzyl)-guanidine

Benzoyl chloride (1.01 g, 0.00712 mole) was added to 1-cyano-1,3-di-(3,4-dichlorobenzyl)-guanidine (2.86 g, 0.00712 mole) in pyridine (25 ml) at 5° and the solution was allowed to stand overnight. The solution was diluted with ether (200 ml), washed with 1 *N* hydrochloric acid (3×200 ml) and with water (2×100 ml). The crude product, m.p. 173–180°, separated at the interface, yield 1.0 g (28%). Repeated recrystallization from methanol raised the melting point of the monobenzoylated derivative to 200–201°, yield 0.50 g (14%). Anal. Calc. for $C_{23}H_{16}Cl_4N_4O$: C, 54.56; H, 3.19; Cl, 28.02; N, 11.07. Found: C, 54.53; H, 3.26; Cl, 28.09; N, 11.18.

1,3-Di-(3,4-dichlorobenzyl)-5-(4-nitrobenzyl)-isomelamine

A solution of 1-cyano-1,3-di-(3,4-dichlorobenzyl)-guanidine (0.75 g, 0.00187 mole) and 4-nitrobenzylcyanamide (0.33 g, 0.00187 mole) in methanol (150 ml) was allowed to stand at 25° for 6 days. The solution was evaporated, and the residue was crystallized from ether (25 ml) to give 0.50 g of a gummy solid. Suspension of the crude product in ether (50 ml) at 5° for 1 week gave the product melting at 170–172°, yield 0.22 g (20%). Recrystallization from methanol raised the melting point to 177–179°. Anal. Calc. for $C_{24}H_{19}Cl_4N_7O_2$: C, 49.76; H, 3.30; Cl, 24.49; N, 16.93. Found: C, 49.90; H, 3.38; Cl, 24.07; N, 16.67.

ACKNOWLEDGMENT

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CONSTITUTION OF A 4-*O*-METHYLGLUCURONOXYLAN FROM THE WOOD OF TREMBLING ASPEN (POPULUS TREMULOIDES MICHX.)[1]

J. K. N. JONES, C. B. PURVES, AND T. E. TIMELL

ABSTRACT

A 4-*O*-methylglucuronoxylan has been isolated in almost quantitative yield from the wood of trembling aspen (*Populus tremuloides* Michx.). The hemicellulose was electrophoretically homogeneous and had a number-average degree of polymerization of 212. Partial hydrolysis gave D-xylose, galacturonic acid, 4-*O*-methylglucuronic acid, and 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylopyranose. Methanolysis and hydrolysis of the fully methylated polysaccharide, which contained 108 xylose residues per average molecule, gave 2-*O*-methylxylose, 2,3-di-*O*-methyl-D-xylose, 2,3,4-tri-*O*-methyl-D-xylose, and methyl 2-*O*-(2,3,4-tri-*O*-methyl-α-D-glucopyranosyluronic acid)-3-*O*-methyl-D-xylopyranoside in a mole ratio of 0.2:95:1:11. It is concluded that the hemicellulose contained a linear framework of approximately 200 (1 → 4)-linked β-D-xylopyranose residues and that, on the average, every ninth xylose unit carried a (1 → 2)-linked 4-*O*-methyl-α-D-glucuronic acid residue directly attached to the xylan backbone. The polysaccharide is evidently similar to the 4-*O*-methylglucuronoxylans occurring in the wood of all arborescent angiosperms so far investigated.

The xylan present in trembling aspen was the first wood hemicellulose to be subjected to detailed structural investigations. Jones and Wise (1) on hydrolysis of aspen wood obtained several monosaccharides, xylobiose, xylotriose, D-galacturonic acid, 4-*O*-methyl-D-glucuronic acid, and 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylopyranose, all of which were unequivocally identified. Milks and Purves (2) isolated from the same wood four fractions containing various amounts of hexuronic, 4-*O*-methylglucuronic and (1 → 4)-linked xylose residues. Partial hydrolysis yielded a crystalline aldotriuronic acid containing one 4-*O*-methylglucuronic acid and two xylose residues. The polysaccharide appeared to be extensively branched and was considered to possess non-reducing end groups of hexuronic acid rather than xylopyranose residues.

Jones, Merler, and Wise (3), on direct extraction of aspen wood with alkali, obtained a hemicellulose fraction in a yield of 19%. This material had a specific rotation of −75° and contained 1.7% methoxyl and 9.2% uronic anhydride groups. Hydrolysis yielded mostly xylose in addition to minor amounts of hexoses, arabinose, and rhamnose. On electrophoresis, the material was resolvable into two components, while on enzymic hydrolysis, 20% was enzyme-resistant. On the basis of results obtained by the methylation technique, the authors concluded that the xylose-containing hemicelluloses consisted of (1 → 4)-linked xylose residues to which were attached, through positions 2 and 3, end groups of L-arabinofuranose and side chains terminated by uronic acid residues. It was pointed out that the acid side chains might contain both glucose and xylose residues, since 2,3,6-tri-*O*-methyl-D-glucose and 3,4-di-*O*-methyl-D-xylose were obtained on hydrolysis of the methylated polysaccharide.

All other xylans so far isolated from the wood of arborescent angiosperms have been found to consist of a linear framework of (1 → 4)-linked β-D-xylose residues, approximately every tenth of which, on the average, carried a (1 → 2)-linked single, terminal side chain of a 4-*O*-methyl-α-D-glucuronic acid residue (4–6). While L-arabinofuranose residues are evidently integral parts of most softwood xylans (7, 8), no such residues have been detected in any hardwood xylan with the exception of that isolated from aspen wood.

[1]*Manuscript received December 5, 1960.*
Contribution from the Department of Chemistry, Queen's University, Kingston, Ontario, the Pulp and Paper Research Institute of Canada, and the Department of Chemistry, McGill University, Montreal, Quebec.

Can. J. Chem. Vol. 39 (1961)

The rather exceptional characteristics of the aspen xylan prompted the present reinvestigation of this hemicellulose. Another aim was to establish the degree of branching and the average length of the xylan backbone, properties which had not been dealt with in the earlier studies.

RESULTS

Aspen wood meal was delignified by alternate treatments with gaseous chlorine and alcoholic monoethanolamine (9), a procedure which, when applied to hardwoods, appears to cause no degradation of the polysaccharides (10). The resulting holocellulose was extracted with aqueous potassium hydroxide (11) to give a product in a yield of 21.4% of the wood. Besides xylose and uronic acids, this material also contained glucose, mannose, and arabinose residues. Complexing with copper lowered the yield to 15.0%, eliminating all hexose- and arabinose-containing polysaccharides. When the wood was directly extracted with aqueous potassium hydroxide, a product was obtained in a yield of 18.1% which gave only xylose and uronic acids on hydrolysis.

The yields and properties of the three products isolated are summarized in Table I.

TABLE I

Yields and characteristics of aspen hemicelluloses

Source	Yield, %*	$[\alpha]_D$†	Xylose per uronic acid calc. from:		
			Methoxyl	Uronic anh.	Equiv. wt.
Wood (A)	18.1	−81°	8.7	9.3	8.6
Holocellulose (B)	21.4	−75°	9.5	7.5	7.5
Copper complex (C)	15.0	−78°	9.5	8.2	7.1

*Based on extractive-free wood.
†In 10% aqueous sodium hydroxide.

It is evident that they differed little in specific rotation or chemical composition and that they contained on the average nine xylose units per *O*-methylglucuronic acid residue. The yields obtained are comparable to the *O*-methylglucuronoxylan content, 19%, calculated from the amount of xylose residues in the wood. The hexose and arabinose residues in the material obtained from the holocellulose were probably not integral parts of the glucuronoxylan since they could be eliminated by complexing with copper and did not appear when the wood was extracted without prior delignification.

The hemicellulose, on boundary electrophoresis, gave only one mobile peak which was very distinct, thus indicating the homogeneity of the material. On oxidation with periodate, the expected 9 moles of oxidant were consumed per repeating unit of the polysaccharide. Attempts to determine the weight-average molecular weight of the polymer by light scattering measurements (12) failed, the value obtained, 200,000, probably being a result of molecular association (13). Osmotic pressure measurements with the acetate derivative (14) gave a number-average degree of polymerization of 212.

A portion of the hemicellulose was partially hydrolyzed and the hydrolyzate was resolved on a column of anion exchange resin into a neutral and an acidic portion. The former crystallized and was identified as D-xylose. The sugar acids were separated on a charcoal–Celite column (15) by gradient elution. Two of the acids obtained were probably galacturonic acid, presumably derived from contaminating pectic material, and 4-*O*-methylglucuronic acid. A third acid had a methoxyl content and an equivalent weight

corresponding to that of an *O*-methylhexuronosyl pentose. This compound was identified through the crystalline tetraacetate of its methyl ester methyl glycoside (16) as 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylopyranose, the same aldobiouronic acid as that first characterized by Jones and Wise (1).

Another portion of the hemicellulose was methylated to completion, first according to Haworth and subsequently by the procedure of Kuhn and co-workers (17). The fully methylated polysaccharide, $[\alpha]_D$ −67°, contained 108 xylose residues as estimated by osmotic pressure measurements, indicating that degradation had occurred during the methylation. The methylated hemicellulose was subjected to methanolysis under conditions known to effect no cleavage of aldobiouronic acids. The resulting mixture of glycosides was resolved into an acidic and a neutral portion on an anion exchange resin. The acid fraction, which contained only one component, was esterified and reduced with lithium aluminum hydride to yield a crystalline, partly methylated glycoside which was identified as methyl 2-*O*-(2,3,4-tri-*O*-methyl-α-D-glucopyranosyl)-3-*O*-methyl-α,β-D-xylopyranoside (18, 19).

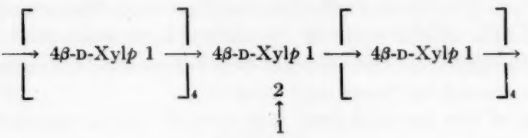
The neutral portion was hydrolyzed to a mixture of reducing sugars which was resolved by gradient elution on a charcoal–Celite column. The three fractions obtained were 2-*O*-methyl-D-xylose, 2,3-di-*O*-methyl-D-xylose, and 2,3,4-tri-*O*-methyl-D-xylose. Paper electrophoresis of the di-*O*-methyl fraction indicated the absence of any 3,4-di-*O*-methyl-D-xylose. A minor portion of the mixture was resolved by paper chromatography and subjected to quantitative analysis (20), giving a molar ratio of 0.2:95:1 for the mono-, di-, and tri-*O*-methylxyloses.

DISCUSSION

The large quantity of 2,3-di-*O*-methyl-D-xylose obtained, in conjunction with the high negative rotation of the original and the methylated hemicelluloses, showed that the xylose residues in the backbone of the macromolecule were linked together by β-(1 → 4)-glycosidic bonds. Methanolysis of the methylated xylan produced no monouronic acids and the partly methylated aldobiouronic acid was unsubstituted at C_4 in the xylose moiety. These facts, together with the absence of any 3,4-di-*O*-methylxylose in the neutral portion, showed that the xylose residues were attached *directly* to the (1 → 4)-linked xylan framework. The constitution of the aldobiouronic acid showed that the side chains were composed of (1 → 2)-linked 4-*O*-methyl-α-D-glucuronic acid residues.

The non-reducing end groups of the polysaccharide undoubtedly gave rise to the 2,3,4-tri-*O*-methyl-D-xylose. The results indicated that 107 xylose residues were present per non-reducing end group while the average macromolecule contained 108 such residues. Only one non-reducing end group was therefore present per average molecule and the xylan framework was accordingly linear. The very small quantity of mono-*O*-methylxylose isolated had probably been formed by demethylation during degradation of the methylated hemicellulose.

From the evidence adduced it is now possible to suggest a tentative structure for the 4-*O*-methylglucuronoxylan present in the wood of trembling aspen. This hemicellulose contains slightly more than 200 β-D-xylopyranose residues linked together by (1 → 4)-glycosidic bonds to a linear framework. Every ninth xylose residue, on the average, carries a single, terminal side chain of a 4-*O*-methyl-α-D-glucuronic acid residue attached to C_2. The acid groups are probably distributed at random along the macromolecules (14). In analogy with similar hemicelluloses present in two species of birch (21, 22), the aspen xylan probably contains *O*-acetyl groups in its native state.



4-*O*-Me-α-D-G*p*A

The xylan present in aspen wood is evidently very similar to the 4-*O*-methylglucuronoxylans previously isolated from the wood of various species of arborescent angiosperms, including American beech (*Fagus grandifolia*) (23), European beech (*F. sylvatica*) (24), Finnish birch (*Betula verrucosa*) (21, 25), white birch (*B. papyrifera*) (26), yellow birch (*B. lutea*) (27), white elm (*Ulmus americana*) (28), and sugar maple (*Acer saccharum*) (29, 30). It is also similar to the 4-*O*-methylglucuronoxylan occurring in the inner bark of white birch (31). The presence of fewer acid side chains and the absence of terminal L-arabinofuranose residues are the main characteristics in which these polysaccharides differ from the xylans present in the wood of most gymnosperms.

EXPERIMENTAL

All specific rotation are equilibrium values and were determined at 20° C. Melting points are corrected. Evaporations were carried out *in vacuo* at 40–50° C.

Paper Chromatography

Solvents (v/v) used for separating the sugars were (A) ethyl acetate – pyridine – water (8:2:1), (B) ethyl acetate – acetic acid – water (9:2:2), and (C) butanone–water (90:10). Separations were carried out on Whatman No. 1 filter paper. Paper electrophoresis was carried out on Whatman No. 3 MM filter paper in a 0.05 *M* borate buffer at 700 v for 4 hours. The spray reagent was *o*-aminodiphenyl. The same reagent was used for quantitative sugar analyses (32).

Composition of the Wood

Aspen wood (33), 40–60 mesh, was exhaustively extracted with ethanol–benzene (1:2). Analyses according to standard methods gave the following analytical data: cellulose (53.3%), pentosan (18.9%), lignin (16.3%), acetyl (3.4%), ash (0.2%), uronic anhydride (3.3%), residues of galactose (0.8%), glucose (57.3%), mannose (2.3%), arabinose (0.4%), and xylose (16.0%).

Isolation of Hemicelluloses

A. From wood.—Extractive-free wood meal (361 g) was treated with 24% (w/w) aqueous potassium hydroxide (2 liters) in an atmosphere of nitrogen at room temperature for 7 hours. The filtrate and washings were poured into four times their volume of ethanol containing an excess of acetic acid, cooled to 0° C. The precipitate formed was recovered by filtration and washed in succession with 70% ethanol, ethanol, and petroleum ether (b.p. 30–60° C). Drying *in vacuo* at room temperature gave a white powder (65.2 g, 18.1% of the wood), $[\alpha]_D$ −81° (*c*, 1.0 in aqueous 10% sodium hydroxide). Anal: OCH_3, 2.33%; uronic anhydride, 12.4%; equiv. wt., 1330. All analyses were carried out with the free acid form of the hemicellulose.

B. From chlorine holocellulose.—Wood meal (765 g) was subjected to three treatments with gaseous chlorine in ice water, followed by extraction with alcoholic monoethanolamine according to the procedure of Timell and Jahn (9). Yield: 692 g, 90.5% of the wood.

The holocellulose (688 g) was exhaustively extracted with 24% aqueous potassium hydroxide as described above to yield a white powder (163 g, 21.4% of the wood), $[\alpha]_D$ −75°. Anal: K salt: Potassium as potassium sulphate, 6.30%, corresponding to 8.7 xylose residues per acid group. Free acid: OCH_3, 2.10%; uronic anhydride, 15.0%; equiv. wt., 1170. Residues of glucose, 1.3%; mannose, 1.7%; arabinose, 1.2%; xylose, 82.4%; 4-*O*-methylglucuronic acid, 13.4%. Extraction of the solid residue with 17.5% aqueous sodium hydroxide to which 4% of boric acid had been added gave a crude glucomannan (20.6 g, 2.7% of the wood) containing 15% of *O*-methylglucuronoxylan, corresponding to 0.4% of the wood.

C. By copper complexing of xylan B.—A portion of xylan B (50 g) was dissolved in 5% aqueous sodium hydroxide (2 liters) and Fehling's solution (1300 ml) was added. The blue precipitate was washed with water until it started to swell, when it was decomposed with ice-cold 0.1 *N* hydrochloric acid in a Waring blendor. The aqueous mixture was poured into 15 liters of ethanol and the precipitate was washed five times on the centrifuge with 80% aqueous ethanol. The material, which was still green, was treated with aqueous ethylenediaminetetraacetic acid for removal of the last traces of copper salts. After neutralization with sodium bicarbonate, the solution (2 liters) was poured into ethanol (10 liters) to give a granular precipitate and a blue solution. The solids were recovered in the usual way and dried to give a white powder (35 g, 15.0% of the wood), $[\alpha]_D$ −78°. Anal: OCH_3, 2.10%; uronic anhydride, 13.9%; equiv. wt., 1130. Hydrolysis and subsequent examination by paper chromatography indicated the presence of uronic acid and xylose only.

Electrophoresis of the Hemicellulose

Free-boundary electrophoresis was carried out with a 2% solution of xylan A in an aqueous solution containing 7.5 g of boric acid and 4.0 g of sodium hydroxide per liter. The apparatus was a Spinco Model H instrument and photographs were taken at three stages of migration. Only one mobile peak was observed.

Determination of the Molecular Weight of the Hemicellulose

Attempts to determine the weight-average molecular weight of the polysaccharide by light scattering measurements with 0.2–1.0% solutions in dimethyl sulphoxide (12) failed. The value obtained, 200,000, was deemed to be the result of fortuitous molecular association.

A portion of the hemicellulose was converted to the fully substituted acetate derivative as described elsewhere (14) to give a product (4.0 g) containing 37.1% acetyl (34). Osmotic pressure measurements were carried out with a 9:1 mixture of chloroform and ethanol (v/v) at 30° C and with Stabin–Immergut (35) osmometers provided with gel cellophane membranes. The results obtained (Table II) gave a number-average molecular weight of 51,400, corresponding to 212 xylose residues in the xylan framework of the average molecule.

Periodate Oxidation of the Hemicellulose

Samples of hemicellulose (A, acid form, 150 mg) were shaken in the dark at 30° C for various lengths of time with 50 ml of 0.05 *M* sodium metaperiodate. The consumption of oxidant was determined by the excess arsenite method. The following moles of periodate were consumed per repeating unit (1378) of the polysaccharide during the first eight days: 1 (9.1), 3 (9.3), 4 (10.2), 6 (10.9), and 8 (11.3). Extrapolation to zero concentration gave a consumption of 9.0±0.1 moles of oxidant per repeating unit.

TABLE II
Osmometry data

Acetylated hemicellulose		Methylated hemicellulose	
*w**	*h/w*†	*w*	*h/w*
2.435	0.502	2.575	1.390
2.029	0.513	2.063	1.364
1.644	0.527	1.675	1.301
1.401	0.518	1.386	1.324
1.125	0.470	1.170	1.329
0.853	0.527	0.759	1.300
0	0.50	0	1.27

*Concentration in g/kg solution.
†Osmotic height in cm solution.

Hydrolysis of the Hemicellulose

The hemicellulose (B, 80 g) was partially hydrolyzed as described earlier (16). After treatment with Amberlite IR-120 exchange resin (acid form), the solution (200 ml) was resolved into a neutral and an acid portion on a column with Dowex 1-X4 exchange resin (acetate form) (16). The neutral sugar fraction contained only D-xylose, m.p. and mixed m.p. 147.5–149° C, $[\alpha]_D$ +20.6° (*c*, 5.0 in water). The dibenzylidene dimethyl acetal derivative (36) had m.p. and mixed m.p. 211–212° C.

The acid fraction contained three sugar acids as indicated by paper chromatography (solvent B). A portion of the mixture (5 g) was added to the top of a Darco G-60 charcoal–Celite (15) column (1:1, 5.5×70 cm). Gradient elution was carried out with aqueous ethanol (37, 38) to give three fractions, corresponding in rate of movement on the paper chromatogram to galacturonic acid (900 mg), 4-*O*-methylglucuronic acid (500 mg), and an aldobiouronic acid (2.3 g). Anal. Calc. for $C_{12}H_{20}O_{11}$:OCH_3, 9.1%; equiv. wt., 340. Found: OCH_3, 9.5%; equiv. wt., 380; $[\alpha]_D$ +100° (*c*, 1.1 in water). The white solid had an infrared diagram identical with that of an authentic specimen of 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylopyranose (7).

A portion of the aldobiouronic acid was converted to the methyl ester methyl glycoside of the tetraacetate derivative (16), as described earlier (27). Recrystallization from ethanol gave crystals, m.p. and mixed m.p. 199–200° C, $[\alpha]_D$ +100° (*c*, 1.3 in chloroform). Anal. Calc. for $C_{22}H_{32}O_{15}$: C, 49.2%; H, 6.02%; OCH_3, 17.3%. Found: C, 49.6%; H, 5.85%; OCH_3, 17.0%. The infrared spectrum of the compound was indistinguishable from that of an authentic sample of methyl 2-*O*-[methyl(2,3-di-*O*-acetyl-4-*O*-methyl-α-D-glucopyranosyl)uronate]-3,4-di-*O*-acetyl-D-xylopyranoside (16).

Methylation of the Hemicellulose

Hemicellulose (A, 24.0 g) was subjected to three methylations with dimethyl sulphate and sodium hydroxide as described earlier (27) to give a white powder (24.7 g). This product was further methylated according to Kuhn and co-workers (17) with methyl iodide, silver oxide, and dimethyl formamide in the presence of Drierite. The product was recovered in the usual way (17) to yield a white, fluffy material (21.5 g), $[\alpha]_D$ −67° (*c*, 2.0 in chloroform). Anal. Calc. for a product containing nine xylose and one 4-*O*-methylglucuronic acid residue: OCH_3, 39.2%. Found: OCH_3, 38.7%.

Methanolysis of the Methylated Hemicellulose and Identification of the Acidic Component

The methylated polysaccharide (15.0 g) was boiled under reflux with 0.8 *N* methanolic hydrogen chloride (650 ml) in the presence of some Drierite for 8 hours. The solution was

neutralized with silver carbonate, filtered through Celite, and evaporated to dryness to give a sirup (15.2 g) which was kept in aqueous barium hydroxide at 60° C for 2 hours. Barium hydroxide was removed by treatment with solid carbon dioxide followed by filtration through Celite. The aqueous solution was treated with Amberlite IR-120 exchange resin (acid form), filtered, and evaporated to a sirup (13.0 g). An aqueous solution of the latter was added to the top of a column with Dowex 1-X4 exchange resin (acid form) (8×20 cm). A mixture of neutral glycosides (10.1 g) was eluted with water (6 liters) after which an acid fraction (2.5 g) was recovered by elution with 30% aqueous acetic acid (4 liters).

The acid glycoside (2.5 g) was re-esterified by boiling with methanolic hydrogen chloride, dissolved in dry tetrahydrofuran (100 ml), and reduced with lithium aluminum hydride (3.0 g) (39). The product was recovered in the usual way to give a sirup (2.0 g) which crystallized spontaneously on evaporation from ethyl acetate. Two recrystallizations from ethyl acetate yielded pure crystals, m.p. and mixed m.p. 165–166° C, $[\alpha]_D$ +91° (*c*, 1.5 in water). Anal. Calc. for $C_{16}H_{30}O_{10}$: C, 50.3%; H, 7.92%; OCH_3, 40.6%. Found: C, 49.7%; H, 8.60%; OCH_3, 40.6%. The infrared spectrum of the compound was identical with that of an authentic specimen of methyl 2-*O*-(2,3,4-tri-*O*-methyl-α-D-glucopyranosyl)3-*O*-methyl-α,β-D-xylopyranoside (27).

Separation and Identification of the Neutral Sugars

The mixture of neutral glycosides eluted from the exchange resin was hydrolyzed with *N* sulphuric acid to give a pale yellow sirup (9 g). A portion of the mixture of reducing sugars (700 mg) was added to the top of a column (3.5×60 cm) containing a 1:1 mixture of Darco G-60 charcoal and Celite. The sugars were separated by gradient elution with 4 liters each of 5% aqueous ethanol → 20% ethanol and 20% ethanol → 40% ethanol. Three fractions were obtained in duplicate experiments in the order given below.

2-O-Methylxylose.—This fraction (2 mg) on electrophoresis had a rate of movement identical with that of 2-*O*-methylxylose.

*2,3-Di-O-methyl-*D*-xylose.*—The main fraction (500 mg) crystallized spontaneously. Two recrystallizations from ethyl acetate – petroleum ether gave crystals with m.p. 94–95° C (40), $[\alpha]_D$ −20° (2 minutes) → +22° (equilibrium, *c*, 2.0 in water). Anal. Calc. for $C_7H_{14}O_5$: OCH_3, 34.8%. Found: OCH_3,3 4.8%. The 2,3-di-*O*-methyl-*N*-phenyl-D-xylopyranosylamine had m.p. and mixed m.p. 124–125° C (41).

*2,3,4-Tri-O-methyl-*D*-xylose.*—This fraction (7 mg), $[\alpha]_D$ +20° C (*c*, 0.5 in water), could not be induced to crystallize. Its rate of movement on the paper chromatogram (solvent C) and its infrared spectrum were identical with those of an authentic sample of 2,3,4-tri-*O*-methyl-D-xylose.

Quantitative Analysis of the O-Methylxyloses

The mixture of neutral *O*-methylxyloses was resolved on filter papers with solvent C. The appropriate strips were excised and eluted with water. The concentration of the sugar solutions were determined by a recent modification of the phenol – sulphuric acid procedure of Smith and his co-workers (20, 42). The reagents used were 3% phenol in water (1 ml), concentrated sulphuric acid (5 ml), and sugar solution (1 ml). The following concentrations in mg/ml corresponded to one absorbance unit (10% transmittance): 2-*O*-methylxylose (118), 3-*O*-methylxylose (63), 2,3-di-*O*-methylxylose (139), and 2,3,4-tri-*O*-methylxylose (87). Paper blanks were analyzed for each compound and the absorbance obtained was deducted from that of the sugar. The average ratio between mono-, di-, and tri-*O*-methylxyloses from several analyses was 0.5:95:1.

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THE STRUCTURE AND RESISTANCE TO METHYLATION OF 1,2-β-GLUCANS FROM SPECIES OF AGROBACTERIA[1]

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ABSTRACT

The structures of extracellular polysaccharides produced by the plant pathogens *Agrobacterium tumefaciens* (2 strains), *A. rhizogenes*, *A. radiobacter*, *A. rubi*, *A. gypsophilae*, and *A. pseudotsugae* were investigated. Polymeric material was formed in good yield from all organisms except *A. gypsophilae*. Periodate oxidations of the polysaccharides and their sodium borohydride reduction products indicated linear 1,2-glucopyranose structures for *A. tumefaciens* (2 strains), *A. rhizogenes*, *A. radiobacter*, and *A. rubi* polymers. Optical rotational considerations led to the assignment of β-configurations. The linear structure for the *A. radiobacter* polysaccharide was confirmed by the isolation, after partial acid hydrolysis, of five 1,2-linked oligosaccharides with chain lengths of two to six. Periodate and chromatographic data indicated that the polysaccharide from *A. pseudotsugae* contained glucose and galactose and 1,6- as well as 1,2-glycopyranose links. It is noteworthy that the polysaccharides from the genus *Agrobacterium* are shown to be mainly 1,2-β-glucans, a structure not yet found in other genera.

Treatment of linear 1,2-β-glucans from *A. tumefaciens*, *A. radiobacter*, and *A. rhizogenes* with sodium hydroxide – dimethyl sulphate, followed by silver oxide in methyl iodide – dimethyl formamide, did not cause full methylation. Some of the 3-hydroxyl groups were not substituted and possible reasons for this unreactivity are discussed.

Hassid and co-workers (1) have shown that *Agrobacterium tumefaciens* (*Phytomonas tumefaciens*) produces in artificial media a polysaccharide consisting mainly of 1,2-D-glucopyranose units joined with the β-configuration (2). It has now been found that several other species of phytopathogenic Agrobacteria form polysaccharides of a similar type. The organisms examined were strains of *Agrobacterium tumefaciens* (virulent and non-virulent), *A. rhizogenes*, *A. radiobacter*, *A. rubi*, *A. pseudotsugae*, and *A. gypsophilae*. The latter organism did not form appreciable amounts of polysaccharide. The yields of 2-linked glycans varied from 2 to 4 g per liter of crude material, depending on the run and the species of organism used.

A striking property of the glucan from *A. tumefaciens* was its resistance to complete methylation (1). Attempts in this laboratory to fully methylate the glucans from *A. tumefaciens* (virulent strain), *A. rhizogenes*, and *A. radiobacter* also did not succeed using, as the final step, the Kuhn procedure (3). In each case, after acid hydrolysis of the methylated product, no 2,3,4,6-tetra-*O*-methyl-D-glucose was isolated, the main product being 3,4,6-tri-*O*-methyl-D-glucose. The undermethylated component was 4,6-di-*O*-methyl-D-glucose, indicating, in the light of periodate data described later, unreactivity of the C_3 hydroxyl group. In the methylation of cellulose, starch, and xylan, the 3-hydroxyl groups are substituted less readily than the other positions, and Croon (4) has suggested that this lower activity is due to hydrogen bonding with the ring oxygen of the adjacent pyranose unit (attached by its reducing end). A molecular model (Courtauld) of 2-*O*-β-D-glucopyranosyl-D-glucopyranose, each unit having the C-1 conformation, shows that this type of bonding can take place in a 1,2-β-glucan. The infrared spectrum of the partly methylated 1,2-β-glucan from *A. radiobacter* contained a strongly hydrogen-bonded hydroxyl band with $\Delta\nu_s$ 195 cm^{-1}.* Since the magnitude of $\Delta\nu_s$ varies with the strength of the hydrogen

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**Difference of frequency between hydrogen-bonded and non-bonded hydroxyl absorption bands.*

bond (5) it is noteworthy that methyl α,β-2,4,6-tri-*O*-methyl-D-glucopyranoside had $\Delta\nu_s$ 75 cm^{-1}, showing that the two possible hydrogen bonds between the C_3 hydroxyl group and the C_2 or C_4 methoxyl groups are weaker. However, this evidence does not rule out interaction between the C_3 hydroxyl in the polymer and the glycosidic oxygen at C_2, but a molecular model suggested that this oxygen atom cannot approach the hydroxyl group as closely as the ring oxygen when the pyranose ring is in the C-1 conformation.

The lower reactivity of the C_3 hydroxyl in a 2-linked glucose dimer can be found in work by Rabaté, who methylated kaempferol sophoroside (6) in a conventional way. Acid hydrolysis of the methyl ether yielded as an unidentified product crystals with m.p. 169° C and $[\alpha]_D$ +113° → 62°, constants corresponding to those of 4,6-di-*O*-methyl-D-glucose. However, when 17-L-hydroxyoctadecanoic acid- and 1,8-nonanediol-sophorosides were methylated under vigorous conditions, no undermethylation was noted (7). It is therefore apparent that the low reactivity of the C_3 hydroxyl in these disaccharide derivatives is merely one of degree. Although strongly hydrogen-bonded hydroxyl groups react more slowly than those less bonded, this property alone does not explain the total unreactivity to methylation of some of the C_3 hydroxyl groups in 1,2-β-glucans. Since there is no reason to believe that the strength of the hydrogen bonds should increase during the course of the methylation to a point of total unreactivity of the group, it is now suggested that the C_3 hydroxyl is increasingly hindered sterically by the rest of the molecule as the methylation proceeds until the group is blocked by the increased bulk of the surrounding methoxyl groups.

Sodium periodate oxidation of the 1,2-β-glucans and derivatives from *A. tumefaciens* (virulent and non-virulent), *A. rhizogenes*, *A. radiobacter*, *A. rubi*, and *A. pseudotsugae* provided more complete structural information than the methylation technique. Excepting the product from *A. pseudotsugae*, the polysaccharides each consumed approximately 1.0 molar equivalents of oxidant, and a little formic acid was produced. As would be expected, overoxidation took place over the observed time of 1–8 days excluding any accurate deductions. *A. pseudotsugae* polysaccharide, which contained galactose as well as glucose, consumed oxidant more gradually up to 0.99 mole after 6 days and gave a high initial production of 0.21 mole of formic acid. After oxidation the dialdehyde units in each of the products were reduced with sodium borohydride and the materials obtained were hydrolyzed with acid. Glycerol was the only detectable hydrolysis product, confirming the linear poly-1,2-D-glucopyranose structure for the polysaccharides from the two strains of *A. tumefaciens*, *A. rhizogenes*, *A. radiobacter*, and *A. rubi*. Since *A. pseudotsugae* polysaccharide gave formic acid on periodate oxidation, it must contain some 1,6-linkages also. The low consumption of oxidant compared with formic acid production therefore shows that the polysaccharide must be impure. All of the above-mentioned polysaccharides except the one from *A. pseudotsugae* have specific rotations near −10°, strongly suggesting β-linkages are present. The polysaccharides from *A. tumefaciens* (two strains), *A. rhizogenes*, *A. radiobacter*, and *A. rubi* were first reduced with sodium borohydride to the derived alcohols, which obviated overoxidation of the reduced end-units (8, 9). The observed consumption of periodate was then 1.0 mole per anhydroglucose unit and small yields of formic acid were produced. However, since no tetramethyl glucose was isolated from methylated polymers on hydrolysis the acid formed may not give a true indication of chain length and may only provide evidence to show that there are differences between the polysaccharides from different species.

The polysaccharide from *A. radiobacter* was examined further and evidence was found to show that the glucan contains at least five consecutive linear 1,2-links. Graded acid

hydrolysis of the polymer yielded a homologous series of oligosaccharides detected on paper chromatograms. Cellulose chromatography of the mixture gave six apparently pure components varying from glucose and sophorose (2-*O*-β-D-glucopyranosyl-D-glucose) to a hexasaccharide. Attempts to crystallize these components, their derived alcohols, or their acetates did not succeed except for sophorose and the trisaccharide identified as *O*-β-D-glucopyranosyl(1 → 2)-*O*-β-D-glucopyranosyl(1 → 2)-D-glucose. Periodate oxidation data on the amorphous alcohols obtained by sodium borohydride reduction of the tri-, tetra-, penta-, and hexa-saccharides, was consistent with linear 1,2-glucopyranosyl linkages only being present (Table I). Also, the oxidation products were reduced with sodium

TABLE I

Specific and molecular rotations and sodium periodate oxidation data of oligosaccharides from *A. radiobacter*

	Dimer	Trimer	Tetramer	Pentamer	Hexamer
Reducing oligosaccharides					
Specific rotations (*c*, 1.0, H_2O)	+15°	+11°	+10°	+7.5°	+5°
Molecular rotations	+5100°	+5500°	+6700°	+6300°	+5000°
Oligosaccharide alcohols					
Specific rotations (*c*, 1.0, H_2O)	−17.5°	−15°	−7.5°	+7.5°	+17.5°
Molecular rotations	−6000°	−5150°	−5000°	+6200°	+17,300°
Periodate consumed (moles/mole)	—	6.18 (6)*	7.01 (7)	8.12 (8)	9.25 (9)
Formic acid formed (moles/mole)	—	2.91 (3)*	3.08 (3)	3.00 (3)	3.21 (3)

*Figures in parentheses are the calculated values.

borohydride and hydrolyzed to yield glycerol only, thus confirming this observation. Although there is no evidence that there are α-linkages in the polysaccharide, the molecular rotations of the oligosaccharides (and derived alcohols) are not, as governed by the Freudenberg and Kuhn equation (10), proportional to their chain lengths (Table I), a situation which applies in other homologous series of oligosaccharides. Possibly this unusual behavior is related to the arrangement, which was such as to contribute to the over-all optical rotation of the molecule, of the sugar units in the chain.

Since *Agrobacterium* species are the only organisms that have yet been shown to produce 1,2-β-glucopyranose polysaccharides or to contain this linkage to some degree, this property provides further justification for placing these organisms in a particular genus. Other work, now at an early stage, on the polysaccharides produced by phytopathogenic organisms of the genera *Corynebacterium* and *Xanthomonas* indicates that in the main there are definite regularities in polysaccharide structure within the genera.

EXPERIMENTAL

Solutions were evaporated under reduced pressure using a bath temperature of 50° C and specific rotations were measured at 26° C. *A. tumefaciens*, strains IIBV7 and IIBNV6, were supplied by the Rockefeller Institute; *A. radiobacter*, strain 1000 by Dr. A. W. Hofer; *A. rhizogenes*, strain TR102, and *A. pseudotsugae*, strain TG102, by Dr. M. Starr; and *A. gypsophilae*, strain 13329, and *A. rubi*, strain 13335, by the American Type Culture Collection.

Isolation of Extracellular Polysaccharides of Agrobacteria

Stock cultures were maintained at 25° C on slants containing 1.5% glucose, 1% yeast extract, 0.2% calcium carbonate, and 1.3% agar. Shake cultures were grown in 500-ml

Erlenmeyer flasks containing 100 ml of the above medium, but without the agar (yeast extract was dialyzed to remove high molecular weight material). Flasks were incubated at 25° C on a rotary shaker having an eccentricity of 1/2 in., operated at 240 r.p.m. Larger volumes of culture were produced in stirred fermentors having volumes of 3 liters or 20 liters using the same medium and growing conditions as for the flask cultures. Cells were removed, after 5 days, by centrifugation in the Sharples supercentrifuge. In a 20-liter fermentation the solution was evaporated to 1 liter and ultracentrifuged to remove small amounts of gelatinous material. The supernatant liquid was added to 4 volumes of ethanol to precipitate the polysaccharide, which was then filtered off and washed with ethanol. The yields of crude polysaccharide varied from 2 to 4 g per liter of medium from *Agrobacterium tumefaciens* (virulent and non-virulent strains), *A. radiobacter*, *A. rhizogenes*, *A. rubi*, and *A. pseudotsugae*, which all proved to contain the 1,2-β-glucan structure. The yield from *A. gypsophilae* was less than 1 g per liter and gave galactose, glucose, and an aldobiuronic acid on acid hydrolysis.

The 1,2-β-glucan-containing polysaccharides were further purified for specific rotation and periodate oxidation measurements. Polysaccharides were dissolved in water, the solutions deionized with Amberlite IR-120 and Dowex-1 and then filtered and evaporated to a small volume. The polysaccharides were isolated by ethanol precipitation and in each case dissolved readily in water to yield non-viscous solutions. The only exception was the polysaccharide from *A. pseudotsugae*, which dissolved only with difficulty to form a somewhat viscous solution. This material was also different in having a higher specific rotation than the other polysaccharides (Table II). When polysaccharides from

TABLE II

Component sugars and specific rotations of Agrobacteria polysaccharides and periodate oxidation of the derived reduced polysaccharides

			Time in hours		
			18	42	66
Source of polysaccharide	Component sugars	$[\alpha]_D$ in water	Periodate oxidation of reduced polysaccharide		
A. tumefaciens (virulent)	Glucose	−12° (*c*, 0.1)	1.01*	1.01	—
			(0.08)	—	—
A. tumefaciens (non-virulent)	Glucose	−12° (*c*, 0.1)	0.98	0.98	—
			(0.04)	—	—
A. radiobacter	Glucose	−13° (*c*, 0.1)	0.99	0.99	—
			(0.02)	—	—
A. rhizogenes	Glucose	−19° (*c*, 0.1)	0.98	0.98	—
			(0.08)	—	—
A. rubi	Glucose	−8° (*c*, 0.1)	0.97	1.05	1.06
			(0.12)	(0.13)	(0.14)
A. pseudotsugae	Glucose and galactose	+48° (*c*, 1.0)	—	—	—
			—	—	—

*These figures are molar periodate uptakes and those in parentheses the formic acid produced.

the other organisms were hydrolyzed with *N* sulphuric acid at 100° C overnight only glucose was detected on paper chromatograms and refluxing with 5% methanolic hydrogen chloride overnight yielded methyl α-D-glucopyranoside. The polysaccharide from *A. pseudotsugae* gave galactose as well as glucose on paper chromatograms after hydrolysis in *N* sulphuric acid at 100° C (spray: *p*-anisidine hydrochloride (11); solvent: ethyl acetate:pyridine:water, 10:4:3 v/v).

Sodium Periodate Oxidations of Polysaccharides

Twenty-five cubic centimeters of water containing 25 mg of polysaccharides was treated with 10 cc 0.1 *M* sodium periodate at 26° C. Uptake of oxidant was measured by addition to aliquots of buffered sodium arsenite containing potassium iodide followed by back-titration with iodine. Acid production was estimated by direct titration against sodium hydroxide. With each polysaccharide except that of *A. pseudotsugae* approximately 1.0 mole of periodate per mole of anhydroglucose unit was consumed within 21 hours reaction time; during the next 120 hours a further uptake of 0.1 mole was observed. Formic acid production increased from 0.10 mole/mole to 0.18 mole/mole when measured simultaneously. The *A. pseudotsugae* polysaccharide consumed 0.78 mole/mole of periodate releasing 0.21 mole/mole of formic acid after 21 hours, these values increasing gradually to 0.99 mole/mole and 0.36 mole/mole, respectively after a further 120 hours. After the oxidations were complete the solutions were deionized with Amberlite IR-120 and Dowex-1. The periodate-oxidized polysaccharides (10 mg) were reduced in the aqueous filtrate (20 cc) with sodium borohydride (10 mg) over a period of 3 hours. Excess reductant was destroyed with acetic acid, Amberlite IR-120 was added and, after shaking, the resin was filtered off and the filtrates evaporated to crusts. Boric acid was removed as trimethyl borate by repeated evaporations with methanol. The residues were hydrolyzed by heating in *N* sulphuric acid (5 cc) at 100° C for 3 hours. The acid was then neutralized with barium carbonate, the solutions filtered, and the filtrates evaporated. Paper chromatographic examination (solvent: butanol:ethanol:water, 40:11:19 v/v) of each hydrolyzate using the ammoniacal silver nitrate spray (12) showed that glycerol only was present. For the polysaccharides from *A. tumefaciens* (two strains), *A. rhizogenes*, *A. rubi*, and *A. radiobacter* 1,2-glucopyranose structures were indicated. However, the *A. pseudotsugae* polysaccharide gave a higher proportion of formic acid on periodate oxidation than the other polysaccharide, thus showing that some 1,6-linked glycopyranose units were present also.

Sodium Periodate Oxidations of Borohydride-Reduced Polysaccharides

Since the above described polysaccharides were overoxidized on treatment with aqueous sodium periodate, the reducing ends were modified with sodium borohydride in order to avoid the possibility of overoxidation starting from this site. Samples (1.00 g) of each polysaccharide were reduced with sodium borohydride (100 mg) in water (100 cc). After 6 hours the reaction mixture was worked up (in a similar manner to that described above for the reduction of the periodate-oxidized polysaccharides) to yield solids which were then oxidized with periodate. The same proportions of oxidant was used for the reduced polysaccharides, as in the oxidation of the unmodified polysaccharides. The results of this oxidation are recorded in Table II.

Methylation of Polysaccharides from A. tumefaciens (virulent), A. rhizogenes, and A. radiobacter

A portion of each polysaccharide (5.0 g) was dissolved in water (20 cc), dimethyl sulphate (3.3 cc) was added and the mixture was stirred vigorously. Sodium hydroxide (3.0 g) in water (10 cc) was introduced dropwise at such a rate as to keep the solution slightly alkaline over a period of 30 minutes. Then sodium hydroxide (30 g) in water (60 cc) was poured into the reaction mixture and methylation was effected using dimethyl sulphate which was added, in 11 separate aliquots of 3 cc each, once every 30 minutes. This process was repeated five times on successive days and then the solution was heated at 100° C for 3 hours. After it was cooled, the methylation mixture was neutralized with

dilute sulphuric acid, and then extracted by shaking four times with chloroform. The extract was dried ($MgSO_4$), filtered, and evaporated to a crust (—OCH_3, 35.8% for the *A. radiobacter* polysaccharide derivative).

The partially methylated polysaccharides (yields 3.9 g to 4.2 g) were dissolved in a dimethyl formamide (30 cc) – methyl iodide (30 cc) mixture and shaken vigorously with portions of silver oxide (100 g total), fresh additions of oxide being made when the suspension had become yellow-brown. After 5 days the solutions were filtered and evaporated to brittle crusts (—OCH_3 contents for *A. tumefaciens*, *A. rhizogenes*, and *A. radiobacter* derivatives, 38.7%, 39.8%, and 39.5% respectively). Remethylation of the partially methylated *A. radiobacter* polysaccharide over a further 9 days under identical conditions did not increase the methoxyl content appreciably. The yields of methylated polysaccharides at the end of the Kuhn procedure appeared to be quantitative.

Samples (2.5 g) of the three partially methylated polysaccharides were refluxed in 10% methanolic hydrogen chloride (25 cc) for 16 hours. The acid was neutralized with silver carbonate and the solutions filtered and evaporated. The sirupy products were hydrolyzed on a steam bath in *N* sulphuric acid (10 cc) for 16 hours and after cooling the solutions were neutralized with barium carbonate, filtered, and evaporated. Paper chromatographic examination with the *p*-anisidine hydrochloride spray showed that, with each hydrolyzate, the main product was trimethyl glucose with a relatively smaller quantity of dimethyl glucose. No tetramethyl glucose was detected. Portions of the hydrolyzates (250 mg) were fractionated on cellulose columns using benzene:ethanol:water (500:50:1 v/v) as the mobile phase, which gave a complete separation. The proportion of each sugar was estimated gravimetrically and the percentages present of the dimethyl hexoses (on a molar basis) in the mixtures from *A. tumefaciens*, *A. rhizogenes*, and *A. radiobacter* were 27%, 20%, and 21% respectively. The trimethyl glucoses were converted to 3,4,6-tri-*O*-methyl-D-gluconic acid phenylhydrazide by the method of Haworth and co-workers (13). This derivative from the polysaccharides of *A. tumefaciens* (virulent), *A. rhizogenes*, and *A. radiobacter* melted at 130–131° C, 130° C, and 129–130° C respectively, and the melting points were not depressed on mixing with an authentic specimen. Also, the X-ray diffraction patterns of these phenylhydrazides and known material were identical. The dimethyl components of the mixtures crystallized readily and were recrystallized from ethyl acetate. The constants obtained on the crystals were consistent with 4,6-di-*O*-methyl-D-glucose (14). Examination of the mother liquors showed that no other dimethyl glucose components were present. The product from *A. tumefaciens* (virulent) polysaccharide had m.p. 160–164° C and $[\alpha]_D$ +110° → +64° (*c*, 1.1, H_2O; 18 hours, constant value). Calculated for $C_6H_{10}O_4(OCH_3)_2$: —OCH_3, 29.8%. Found: —OCH_3, 29.3%. The 4,6-di-*O*-methyl-D-glucoses from *A. radiobacter* and *A. rhizogenes* polysaccharides melted at 161–164° C and 161–162° C respectively. The dimethyl glucoses from the three different sources did not have their melting points depressed on intermixture.

Infrared Spectrum of the Partially Methylated A. radiobacter Polysaccharide

The partially methylated polysaccharide was dissolved in dry chloroform as 0.04 *M* and 0.2 *M* solutions. At 27° C both of these solutions showed strong absorption at 3465 cm^{-1} and a weak band at 3660 cm^{-1}, thus giving a $\Delta\nu_s$ of 195 cm^{-1} between hydrogen-bonded and undissociated hydroxyl groups.

A sample of methyl α,β-2,4,6-tri-*O*-methyl-D-glucopyranoside had its infrared spectrum measured under similar conditions. In this case there was a strong band at 3605 cm^{-1} and a very weak one at 3680 cm^{-1} indicating a $\Delta\nu_s$ of 75 cm^{-1}.

Partial Hydrolysis of A. radiobacter Polysaccharide

The polysaccharide (8.75 g) was dissolved in *N* sulphuric acid (200 cc) and the hydrolysis mixture was kept at 70° C for 18 hours. The acid was neutralized ($BaCO_3$), the solution was filtered and evaporated to a syrup which was fractionated on a cellulose column. *n*-Butanol saturated with water eluted glucose (1.62 g); butanol:ethanol:water (4:1:1 v/v) gave a disaccharide (0.79 g) and a trisaccharide (0.37 g); butanol:ethanol:water (8:3:3 v/v) gave a tetrasaccharide (0.49 g); and butanol:ethanol:water (2:1:1 v/v) a pentasaccharide (0.27 g) and a hexasaccharide (0.13 g).

The disaccharide crystallized from methanol–ethanol to yield sophorose with m.p. and mixed m.p. 198–200° C and $[\alpha]_D$ +28° → +16° (*c*, 1.0, H_2O; 18 hours, constant value). The trisaccharide was twice crystallized from aqueous methanol to give *O*-β-D-glucopyranosyl-(1 → 2)-*O*-β-D-glucopyranosyl-(1 → 2)-D-glucose with m.p. 218–223° C and $[\alpha]_D$ +16° → +11° (*c*, 1.0, H_2O; 18 hours, constant value). Calculated for $C_{18}H_{32}O_{16}$: C, 42.9%; H, 6.4%. Found: C, 42.55%; H, 6.7%.

The tetra-, penta-, and hexa-saccharides and their acetates were amorphous as were the derived polyols (by sodium borohydride reduction) and their acetates. The specific and molecular rotations of the five reducing oligosaccharides and their derived alcohols are recorded in Table I. The polyols were oxidized in aqueous sodium periodate and the uptake of oxidant and formic acid production measured after 18 hours (Table I) using the proportions in the oxidation of the reducing polysaccharide described earlier in the paper. The values obtained correspond to the presence of 1,2-linked hexapyranose units only.

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A STUDY OF THE CN EMISSION FROM ACTIVE NITROGEN FLAMES[1]

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ABSTRACT

When organic compounds are added to active nitrogen, the red and violet bands of the CN radical are emitted. The relative intensities of these red bands have been measured for a variety of added compounds, temperatures, and pressures. It is shown that there are at least three processes producing electronically excited CN radicals, two yielding CN($A^2\Pi$) and one yielding CN($B^2\Sigma$). The behavior of the flames indicates that a chain mechanism is involved. Adding ammonia, which does not react with nitrogen atoms, quenches the CN emission and inhibits the consumption of nitrogen atoms. It is concluded that a second reactive species in active nitrogen, possibly metastable $N_2(A^3\Sigma)$ molecules, initiates the reactions which result in light emission.

INTRODUCTION

When organic compounds are added to active nitrogen, a red or lilac flame appears which consists mostly of bands of the red ($A^2\Pi \rightarrow X^2\Sigma$) and violet ($B^2\Sigma \rightarrow X^2\Sigma$) systems of the CN radical (see Fig. 1). Winkler and co-workers have studied the products of the

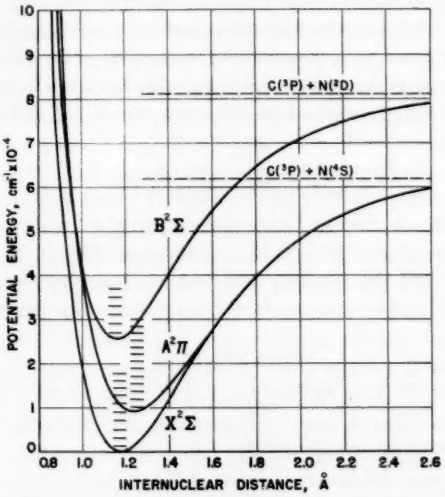

FIG. 1. The Morse potential energy curves for CN. The value 7.53 ev has been used for the dissociation energy of CN, the other constants are from G. Herzberg. Molecular spectra and molecular structure, I. Spectra of diatomic molecules. Van Nostrand, New York. 1950. The low-lying vibrational levels are indicated by the short horizontal lines within each curve.

reactions of active nitrogen with various compounds (1). They have proposed reaction mechanisms which could account for the observed products: however, they only speculate on the nature of the CN emission. Recently Kiess and Broida (2) have systematically studied the spectra of active nitrogen flames using various organic compounds. They concluded that there are at least two different reactions which produce electronically excited CN radicals. The present work was undertaken to learn more of these reactions which are responsible for the CN emission. In order to simplify the investigation as much as possible, only simple compounds have been used.

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EXPERIMENTAL

The active nitrogen was formed in an electrodeless discharge (Deutsche Elektronik, 2500 Mc, 1000 w) operated at 0.6 to 30 mm Hg nitrogen pressure. A minimum of 0.5 second was required for the gas to flow from the discharge through 110 cm of Pyrex tubing (7-mm i.d.) to the reaction zone. The observation cell was a Pyrex tube of 30-mm inner diameter, 40 cm long, with a flat Pyrex window at the end where the active nitrogen stream entered. The organic compounds flowed through a capillary tube into the active nitrogen stream 3 cm before it entered the observation cell. A second capillary 40 cm upstream from the observation cell was used for adding ammonia and hydrogen in the quenching experiments. The pressure was measured by a small McLeod gauge at the exit of the observation cell. A cold trap prevented the products of the reaction from entering the rotary oil pump. All of the glass surfaces between the discharge and the cold trap were treated with a dilute solution of hydrofluoric acid in order to minimize atom recombination on the walls.

The observation cell and the entrance tubing were wrapped with Nichrome wire and asbestos so that spectra at elevated temperatures could be taken. A glass thermometer inside the cell measured the gas temperature. It was also possible to surround the cell with dry ice for low-temperature studies.

Commercially purified nitrogen (Nachgereinigter Stickstoff, Linde's Eismaschinen) was used without further purification. The CCl_4, $HCCl_3$, $HCBr_3$, and BrCN were chemically pure commercial products and were used without further purification. The HCN was produced by dropping concentrated sulphuric acid on NaCN and collecting the evolved gas in a dry-ice trap. The ClCN and $(CN)_2$ were prepared by standard procedures (3). The ClCN was redistilled twice, and only the most volatile half was retained, in order to separate it from the CCl_4 which was used in the preparation. Pressure drops across the flow capillaries were measured with mercury manometers. Gas flows were calculated using the Bernoulli equation for a compressible fluid. Standard viscosities were used when available (4). The viscosities of ClCN, BrCN, and HCN were assumed, for simplicity, to be equal to the viscosity of $(CN)_2$. The smaller capillaries were calibrated empirically by measuring the rate of pressure change in a known volume.

A small condensing lens focused the light from the observation cell onto the entrance slit of a Steinheil Universal Spectrograph which was equipped with $f/5$ glass optics and a plane grating (600 lines/mm). The reciprocal dispersion at the exit slit was 60 Å/mm. The spectrum was scanned by slowly turning the grating. Both entrance and exit slits were 0.5 mm wide, giving a resolution of about 30 Å. The use of glass optics should have resulted in some defocusing of the image as the grating was rotated; however, the effect was small and did not appreciably effect the resolution.

An infrared-sensitive photomultiplier tube (RCA 7102) was placed 4 cm behind the exit slit. A chopper between the cell and the condensing lens modulated the light at about 180 c.p.s. An ordinary 6-v bicycle generator was mounted on the same axis as the chopper to provide a reference signal of the same frequency and with a fixed phase difference compared to the light signal. The signal from the phototube and the reference signal were amplified and compared by a lock-in amplifier, the output going to a 5 mv Siemens strip chart recorder. The phototube was used at dry-ice temperature, and the other usual precautions were taken to minimize the noise level. A Jena OG 5"A" filter, which transmitted only wavelengths longer than 5300 Å, was used at the condensing lens to prevent the second-order blue spectrum from overlapping the red bands. For the very strong flames it was necessary to use neutral filters (Mikro-Siebblech) of 10

and 1.5% transmission in order to prevent overloading the amplifier. A pip marker was used to correlate the rotation of the grating with the strip chart record.

The spectral sensitivity of the system was measured by taking the spectrum of a calibrated Osram tungsten filament bulb, said to have a black-body temperature of 2300° K. A calcium sulphate reflector was used just before the observation cell window to simulate the intensity and position of the flames. Since the present study was more interested in changes in the relative intensities of the bands, the small corrections for the emissivity of tungsten or the reflectivity of calcium sulphate were not made. The system had a maximum sensitivity per quantum at about 7000 Å, which decayed to about 20% of maximum at 10,000 Å and 5% at 11,000 Å. Toward the end of this study the photocathode sensitivity beyond 10,000 Å decayed considerably, making the intensity of the $A(0)$–$X(0)$ band* much less certain.

The areas of the peaks on the strip chart were measured with a compensating planimeter. These areas were divided by the relative sensitivity determined in the calibration in order to obtain the relative band intensities. Since the sensitivity of the photomultiplier did not extend far enough into the infrared to measure all of the CN bands, and since for some of the conditions used in this study the overlapping nitrogen afterglow spectrum was of comparable intensity with the CN bands, only one band was used as a measure of the total emission from a given vibrational level of $A^2\Pi$. The bands used ($A(0)$–$X(0)$, $A(1)$–$X(0)$, $A(2)$–$X(1)$, $A(3)$–$X(1)$, $A(4)$–$X(2)$, $A(5)$–$X(1)$, $A(6)$–$X(2)$, $A(7)$–$X(3)$, $A(8)$–$X(3)$, $A(9)$–$X(3)$, $A(10)$–$X(4)$) were chosen to have a minimum of overlap by other bands. When necessary, corrections were made for overlapping by weak nitrogen afterglow bands. These corrections never amounted to more than 30% of the CN intensity, and were usually much less. Minor corrections were usually made on the spectra to account for slight changes in intensity or in the base line during a run. The emission curves were reproducible to within several per cent of the total emission.

The intensity of a measured band, $A(v')$–$X(v'')$, was converted to the total emission from the level $A(v')$ by using the previously measured intensities of the CN red system (5). The total emissions from the various levels $A^2\Pi(v')$ were then plotted against the vibrational quantum number v' to give an "emission" curve as is shown in Fig. 2. Such

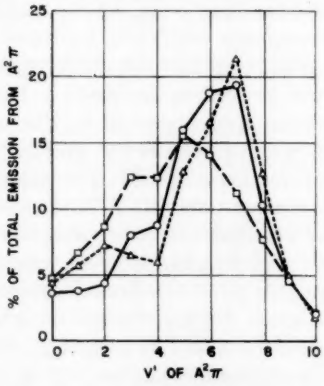


FIG. 2. Emission curves for the CCl_4 – active nitrogen flames. The percentage of the total emission from $A^2\Pi$ which originates in the level $A^2\Pi(v')$ is plotted against v'. Total pressure 3.0 mm Hg, CCl_4 flow 6×10^{-8} mole/sec. Temperature: □ 450° K; ○ 300° K; △ 200° K.

*This abbreviated notation will be used for the transition $A^2\Pi(v'=0) \rightarrow X^2\Sigma(v'=0)$.

a curve represents the relative number of quanta that are emitted from each vibrational level of $A^2\Pi$. If it can be assumed that the electronically excited molecules undergo no deactivating collisions, and that there is a steady-state concentration of excited CN molecules, then the emission curve would also represent the relative number of molecules which are being formed in the various vibrational levels of the $A^2\Pi$ state.

RESULTS

The spectrum of the CCl_4 – active nitrogen reaction was studied for a variety of conditions. For total pressures lower than about 5 mm Hg and CCl_4 flow rates lower than 10^{-7} mole/sec the spectral distribution of the CN red system did not change within the experimental error for changes in pressure (0.6 to 5 mm Hg) or CCl_4 flow.* There did appear to be a slight shift to higher vibrational levels with decreasing temperature as is shown in Fig. 2. At pressures greater than 5 mm and for higher flow rates of CCl_4 the distribution shown in Fig. 2 for 300° K (solid line) began to shift to lower vibrational quantum numbers in a manner which suggested that vibrational relaxation within the $A^2\Pi$ state was becoming important. Thus at 9.3 mm pressure (N_2 flow 2.6×10^{-5} mole/sec) and a CCl_4 flow of 2.4×10^{-8} mole/sec it was possible to obtain an emission distribution similar to that reported by Dixon and Nicholls (5) for similar conditions. Since vibrational relaxation masked the original distribution, further runs were limited to the lower pressures and flow rates.

The $HCCl_3$ flame at room temperature had an emission curve similar in shape to that of the CCl_4 flame, but with a less pronounced peak at $v' = 5$, 6, and 7. The emission curve for a $HCBr_3$ flame also had a peak centered on $v' = 6$ and 7, but it was significantly broader than the peak observed for the CCl_4 flame, especially toward higher vibrational levels. The stronger emission from the 10th vibrational level of $A^2\Pi$ for organic bromide flames has been noted previously (2).

In order to determine the effect of the reaction products on the spectral distribution of the CN emission, a study of the compounds XCN (X = H, Cl, Br, CN) was made. HCN is the main nitrogen-containing compound which is formed in active nitrogen–hydrocarbon reactions (1). The only detectable products of the CCl_4 flame were found to be ClCN and Cl_2 (6), so that it is expected that BrCN would be formed when organic bromides are used. Cyanogen is usually formed in small amounts in most of these reactions.

When HCN was added to active nitrogen, the CN emission was rather weak but the spectrum was very interesting and, therefore, was extensively studied. The distribution of the CN red emission for a HCN – active nitrogen mixture is shown in Fig. 3. A distribution almost identical with that shown in Fig. 3 was observed when ethylene at a flow rate of 1.9×10^{-8} mole/sec was added to active nitrogen at a pressure of 2.3 mm. In contrast to the CCl_4 flame, the relative intensities within the CN red system depended on both the flow rate of HCN and on the total pressure.

The curve of Fig. 3 can be divided up into two peaks. P_1 is arbitrarily defined as the emission from $A^2\Pi(v' = 0, 1, 2)$ and half of the emission from $v' = 3$, and P_2 is the remaining emission from $v' = 3$ and from the higher vibrational levels. It is believed that P_1 is not the result of vibrational relaxation of CN molecules originally formed in $A^2\Pi(5, 6, \text{and } 7)$. The pressure and flow of fuel are below the values mentioned above, at which vibrational relaxation is first apparent in the CCl_4 flame. Also the

*The spectra of reference 2 show a slight shift to lower v' in going from 1 mm to 4 mm Hg pressure. No reason for this disagreement is apparent.

distribution in Fig. 3 is not what is theoretically expected for such a partially relaxed system (7).

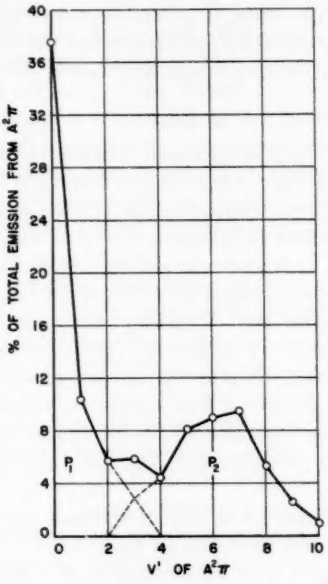
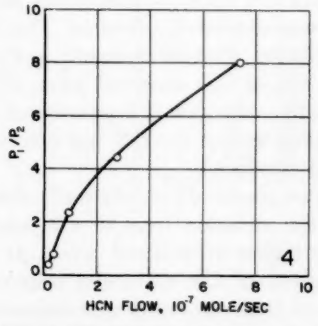


FIG. 3. Emission curve for a HCN – active nitrogen mixture. Pressure 3.3 mm Hg, temperature 300° K, HCN flow 8×10^{-8} mole/sec.

While holding the active nitrogen flow constant (total pressure 3 mm) and increasing the HCN flow, the ratio P_1/P_2 varied as is shown in Fig. 4. Adding HCN did not decrease the intensity of the nitrogen afterglow initially: however, there were gradual changes in intensity whenever hydrogen-containing compounds were added, probably because of changes in wall conditions. Therefore, it is necessary to compare the ratio of intensities within a given spectrum. The change of the inverse ratio, P_2/P_1, with nitrogen flow is shown in Fig. 5. It appears that in the limit of small HCN flow the ratio P_1/P_2 approaches



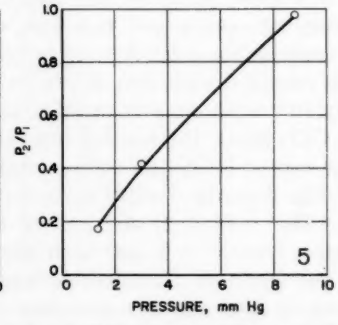


FIG. 4. The ratio P_1/P_2 for constant total pressure (3.0 mm Hg), constant temperature (300° K), and varying HCN flow.

FIG. 5. The ratio P_2/P_1 for constant HCN flow (9×10^{-8} mole/sec), constant temperature (300° K), and varying total pressure.

zero, while in the limit of low active nitrogen flow (or low pressure) the ratio P_2/P_1 approaches zero.

When cyanogen was added to active nitrogen the emission from the lowest vibrational levels of $A^2\Pi$ was comparable, both in intensity and spectral distribution to that observed with HCN. But the emission from the 5th, 6th, and 7th vibrational levels (P_2) was approximately 10 times weaker. In spite of this, the ratio of intensities $A(1)$–$X(0)/A(8)$–$X(3)$ showed the same shape curve as was found for P_1/P_2 with HCN (Fig. 4). Although small additions of cyanogen (10^{-8} mole/sec) did not reduce the normal afterglow intensity, larger flow rates quenched it almost completely, in agreement with previous work (8).

In contrast to the weak CN emission associated with HCN and $(CN)_2$, both ClCN and BrCN gave very bright flames, much brighter even than the CCl_4 flames. At 3 mm nitrogen pressure the reaction was so rapid that the flame was confined to the 7-mm tubing between the capillary and the observation cell. For this confined flame no afterglow could be observed downstream, and a brown deposit rapidly built up on the glass walls in contact with the flame. Thus it was necessary when studying these compounds to introduce the fuel capillary directly into the cell volume. When this was done, a roughly spherical flame formed, from 1 to 3 cm in diameter depending on the flow conditions. The distributions of CN emission from such ClCN and BrCN flames are shown in Fig. 6. Changing the ClCN or BrCN flow only changed the size of the flame, without

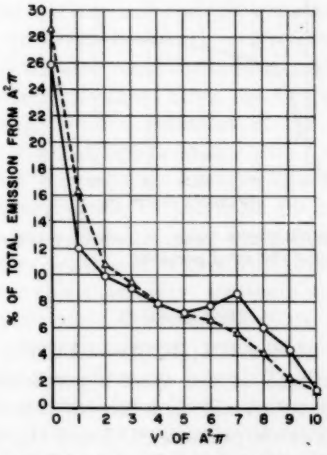


FIG. 6. Emission curves at 3.0 mm Hg pressure and 300° K, for ○ ClCN and △ BrCN flames.

changing the relative intensities of the emission. Runs at 1.3 mm nitrogen pressure, for which there was no bright flame but a diffuse emission throughout the cell volume, gave emission curves equal, within experimental error, to those shown in Fig. 6.

Since it is known that ammonia quenches certain of the first positive bands in the nitrogen afterglow faster than others (9), it was decided to test its quenching effect on the CN emission. With the nitrogen flow rate 1.4×10^{-5} mole/sec, the pressure in the cell was 3.0 mm and the Lewis–Rayleigh afterglow filled the entire system. Addition of 0.2% BrCN gave a bright flame and no afterglow could be observed downstream. Then 0.7% ammonia was added to the active nitrogen stream 40 cm (0.1 second) before it entered the observation cell. This weakened the CN emission by a factor of 62 and

the nitrogen afterglow could once again be observed downstream, although it was not quite as intense as for the pure nitrogen. Increasing the BrCN flow sevenfold increased the total CN emission by less than 10% although it did shift the distribution somewhat to low vibrational levels. Essentially complete quenching of the CN emission occurred for larger ammonia flows. When 0.2% BrCN was added to active nitrogen containing 2% ammonia, there was no change in the relative intensities of the nitrogen first positive bands (i.e., no overlapping CN emission) but the total intensity of the afterglow was reduced by about 20%. Similar quenching by ammonia was observed for the HCN and CCl_4 reactions.

When the filter was not used before the entrance slit, the CN blue system in the second order was superimposed on the red system. For the conditions of this study the emission from the lowest vibrational level of the $B^2\Sigma$ state was much stronger than the emission from higher vibrational levels. The photomultiplier tube was not calibrated in the blue region of the spectrum, but the intensity of the $B^2\Sigma(v' = 0) \rightarrow X^2\Sigma(v'' = 0)$ band, in arbitrary units, divided by the total emission in the red system is plotted in Fig. 7 against the total pressure for a constant flow of CCl_4.

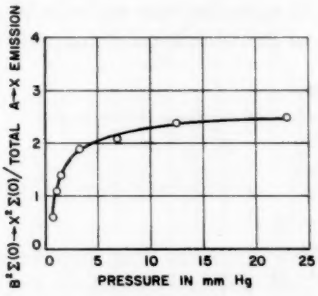


FIG. 7. The intensity of the $B^2\Sigma(0)$–$X^2\Sigma(0)$ band, in arbitrary units, divided by the total emission in the $A^2\Pi$–$X^2\Sigma$ system, plotted against the total pressure.

DISCUSSION

The variation of intensity of the CN($B^2\Sigma(0) \rightarrow X^2\Sigma(0)$) band with total pressure (Fig. 7) was expected on the basis of the spectra previously reported by Kiess and Broida (2). They resolved the rotational structure of this band in a CH_2Cl_2 – active nitrogen flame, and showed that at low pressure (0.15 mm Hg) the blue emission originates almost entirely in those rotational levels of $B^2\Sigma(0)$ which are perturbed by rotational levels of $A^2\Pi(10)$. As the pressure was increased, the emission from the other, non-perturbed rotational levels of $B^2\Sigma(0)$ increased, until approximate rotational equilibrium was attained at 11 mm pressure. This behavior could be interpreted in the following way. The CN molecules are originally formed in the $A^2\Pi$ electronic state, some with 10 quanta of vibration. Because of the mixing of eigenfunctions (10) of the perturbed rotational levels of $A^2\Pi(10)$ and $B^2\Sigma(0)$, transitions between these two states can take place easily for certain J's. If the pressure is high enough, collisions can redistribute the $B^2\Sigma$ molecules among the other nonperturbed rotational levels before the allowed emission of radiation occurs. If one assumes a rapid equilibrium of molecules between the perturbed rotational levels of $A^2\Pi(10)$ and $B^2\Sigma(0)$, then the total emission from $B^2\Sigma(0)$ will depend on the rate of approach to equilibrium between the perturbed and unperturbed rotational levels of $B^2\Sigma(0)$, and thus on the total pressure. This mechanism

cannot explain the "tail bands" of CN, originating from high vibrational levels of $B^3\Sigma$, which sometimes appear in active nitrogen flames (11).

It is now generally accepted that active nitrogen contains nitrogen atoms in their ground state (12, 13). However, there is strong evidence that at least one other reactive species is present. Kaufman and Kelso (14) reported a temperature increase when N_2O, which is known not to react with nitrogen atoms (15), is introduced into active nitrogen. Freeman and Winkler (16) found that ammonia was decomposed by active nitrogen, and their experiments indicated that atomic nitrogen was not responsible. Kistiakowsky and Volpi (17) showed that the atomic nitrogen concentration was not reduced by additions of up to 7% ammonia. Since it was observed in this study that the intense CN emission could be completely quenched by the addition of 2% ammonia, it seems apparent that the second reactive species is important in at least initiating the reaction which leads to the CN emission.

Any of the electronic states of molecular nitrogen which participate in the afterglow mechanism might be the second reactive species. These include the proposed $^5\Sigma$, the $B^3\Pi$, and the $Y^3\Sigma$, which are the upper states of the afterglow emission, and the $A^3\Sigma$, which is the lower electronic state of the emitting molecules (9). Vibrationally excited ground state molecules should also be present since they will be formed by direct recombination of nitrogen atoms and by deactivation of $A^3\Sigma$ molecules. However, the observation that ammonia quenches the CN emission eliminates the possibility that the $^5\Sigma$, $B^3\Pi$, and $Y^3\Sigma$ states are the initiators of the CN emission. The small concentration of ammonia which was used quenches the nitrogen afterglow only slightly. This requires that the concentration of all the electronic states of nitrogen which precede the afterglow emission remain approximately constant. Since the CN emission is reduced drastically by the addition of ammonia, the concentration of the reactive species initiating the CN emission must also be changed significantly.

It has been suggested that ground state nitrogen molecules in high vibrational levels are responsible for the decomposition of ammonia by active nitrogen (18). If this is so, the ammonia molecules must absorb a large amount of the excess vibrational energy (since the ammonia is decomposed) and in this way prevent the reactions which result in the CN emission. To test this possibility, methane was substituted for ammonia in the quenching experiment previously described. Methane was chosen because it does not react significantly with active nitrogen at room temperature (19), and because its efficiency of transferring vibrational energy from N_2O is comparable to that of ammonia (20). The CN emission was reduced by a factor of two when 0.7% methane was added to a BrCN – active nitrogen flame, as compared with a factor of 62 for 0.7% ammonia. If vibrationally excited ground state nitrogen molecules are the second reactive species in active nitrogen, it is difficult to understand this large difference in the quenching effect of ammonia and methane. But because of the complexity of such an energy transfer, this experiment cannot be considered conclusive evidence against the vibrationally excited ground state hypothesis.

Nitrogen molecules in the lowest triplet state, the metastable $A^3\Sigma$, could also be the second reactive species in active nitrogen. Such molecules are certainly being formed in active nitrogen since this is the lower electronic state of the afterglow emission. Also it has been estimated that only 1 out of 20 recombining pairs of nitrogen atoms emits a quantum in the afterglow (21), the other pairs must recombine directly into the $X^1\Sigma$ and $A^3\Sigma$ states. On a statistical basis it is expected that three fourths of these recombining pairs will form $A^3\Sigma$ molecules. Thus approximately 16 molecules are formed in the $A^3\Sigma$ state for every quantum emitted in the Lewis–Rayleigh afterglow.

Several unsuccessful attempts have been made to observe $A^3\Sigma$ state molecules in active nitrogen. Exposures of 60 hours (22) failed to detect emission of the Vegard–Kaplan bands, $A^3\Sigma \rightarrow X^1\Sigma$, or the Lyman–Birge–Hopfield bands, $a^1\Pi \rightarrow X^1\Sigma$, from active nitrogen. But the latter have since been observed with much shorter exposures (13). Worley could find no absorption from the $A^3\Sigma$ state using a 13-m path length in active nitrogen (23), from which he concluded that the concentration of $A^3\Sigma$ was smaller than 10^{10} molecules/cm^3. However, mercury vapor was present in his experiments which might have reduced the concentration of triplet molecules by collisional deactivation.

It can be argued that if $A^3\Sigma$ molecules are the second reactive species, then Freeman and Winkler's estimate (16) that the second species is about one-sixth as abundant as nitrogen atoms is incompatible with the failures to observe the $A^3\Sigma$ state in emission or absorption. However, for the apparatus used in reference 16 the elapsed time between the discharge and the reaction cell was comparable with the radiative lifetime of the $A^3\Sigma$ state (0.026 second or longer (24)), so that metastable molecules formed in the discharge might be responsible for the high concentration of the second reactive species. This interpretation is consistent with their observation that increasing the voltage on the discharge increased the relative amount of the second reactive species (18).

The $N_2(A^3\Sigma)$ molecule could transfer its electronic energy to XCN, exciting it to a repulsive triplet state (I) which would then dissociate to CN and X radicals (II).

$$N_2(A^3\Sigma) + XCN \rightarrow N_2(X^1\Sigma) + XCN^* \quad (I)$$

$$XCN^* \rightarrow X + CN \quad (II)$$

It is known that such transfers in which the total spin is conserved can occur readily (25). The energy available from the triplet state of nitrogen, assuming a transition from $A^3\Sigma(0)$ to $X^1\Sigma(0)$, is 142 kcal/mole. The bond energy of X—CN has been calculated using the recently favored value for D(CN—CN) of 146 kcal (26) and accepted thermodynamic data (27, 28), and is presented in the second column of Table I. The energy necessary to break the X—CN bond and excite the CN radical to the $A^2\Pi(0)$ level (an additional 26 kcal) is shown in the last column in Table I. It is seen that the 142 kcal provided

TABLE I

The bond energies of X—CN (second column); the energies necessary to break the X—CN bond and excite the CN radical to the $A^2\Pi(v' = 0)$ level (third column)

Compound	Bond energy D in kcal	D + 26 kcal
$(CN)_2$	146	172
HCN	130	156
ClCN	107	133
BrCN	94	120

by the transition $A^3\Sigma(0)$–$X^1\Sigma(0)$ is sufficient to break the X—CN bonds, with the possible exception of $(CN)_2$. However, to break the X—CN bond and excite the CN radical to the $A^2\Pi$ state requires more than 142 kcal for HCN and $(CN)_2$. This might explain the weak CN emission associated with these compounds as compared to the more weakly bonded halogen cyanides. If the $A^3\Sigma$ molecules are vibrationally excited when they react, there would be correspondingly more energy available to excite the CN radical.

The decomposition of more complex molecules (at least those whose flames are quenched by ammonia) might occur by a similar mechanism. A transfer of electronic energy from

$N_2(A^3\Sigma)$ to the complex molecule, similar to step (I), could cause an initial bond rupture. Any polyatomic radicals formed should react rapidly with atomic nitrogen, yielding CN compounds or the CN radical directly.

The behavior of the flames observed in this work seemed to indicate that a chain reaction is involved. Sometimes, when adding very small amounts of fuel to the active nitrogen stream, an oscillating flame front would form. This was especially noticeable for the CCl_4 reaction at 80° C. This flame front, which gave the usual bright CN emission, would sometimes pulsate at about 5 c.p.s. with an amplitude of 1 cm. But it could be made to slowly sweep the entire length of the observation cell in an upstream direction once every several seconds, the flame completely disappearing at the window end of the cell before reforming again downstream. In between the passage of the flame the normal afterglow filled the tube, but the passing of the flame weakened it considerably. The effect was very sensitive to changes in fuel flow and total pressure, and was not entirely reproducible from day to day, possibly because of wall conditions. Such a lower concentration limit to a fast reaction is typical of a chain mechanism (29).

The complete consumption of nitrogen atoms in the BrCN and ClCN flames also suggests a chain mechanism. The second reactive species is, at most, one-sixth the concentration of nitrogen atoms (16), and is probably much less in these experiments because of the long flow time between the discharge and the reaction cell. But the removal of the second reactive species by ammonia prevents, essentially completely, the consumption of nitrogen atoms. Such behavior could be explained if the second reactive species initiates a chain reaction which consumes nitrogen atoms.

It was suspected, from the behavior of the CN emission in the HCN – active nitrogen mixtures, that the P_2 peak in Fig. 3 was associated with a reaction of free CN radicals. This possibility was tested by further experiments. It is known that CN radicals will react with molecular hydrogen (30). Therefore, the addition of hydrogen to a HCN – active nitrogen mixture should reduce the CN concentration, and thus the height of the P_2 peak, if the above suspicion is correct. With a total pressure of 3.0 mm and a flow rate of HCN of 2.5×10^{-8} mole/sec, the ratio P_2/P_1 was 1.1. Addition of 1.2×10^{-5} mole/sec of hydrogen between the discharge and the cell increased the total pressure to 3.3 mm and decreased the ratio P_2/P_1 to 0.2, as predicted above. The results of a similar experiment using ClCN instead of HCN are shown in Fig. 8. The response of the photomultiplier at 11,000 Å had decayed considerably by the time this experiment was done, so the intensity of the $A^2\Pi(0)$–$X^2\Sigma(0)$ band was rather uncertain. The two

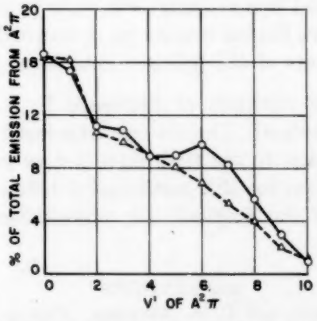


FIG. 8. Emission curves at 300° K. ○ Pressure 3.1 mm Hg, flow ClCN 7×10^{-8} mole/sec. △ Pressure 3.7 mm Hg, flow ClCN 7×10^{-8} mole/sec, flow hydrogen 2.4×10^{-5} mole/sec.

emission curves have been normalized so that the total emission from the lowest three vibrational levels of $A^2\Pi$ is equal. It is seen that the emission from the 5th through 8th vibrational levels is reduced by the addition of hydrogen, and the difference between the two curves has the same shape, within experimental error, as the P_2 peak for the HCN spectrum. It thus appears that the population curve for the ClCN flame shown in Fig. 6 could also be interpreted as consisting of a P_1 and a P_2 peak. But the P_1 peak for ClCN extends to higher vibrational levels than the P_1 peak for HCN.

One possible behavior of the free CN radical would be to catalyze the recombination of nitrogen atoms, as is shown in steps (III) and (IV).

$$N + CN \rightarrow CN_2 \qquad \text{(III)}$$

$$CN_2 + N \rightarrow CN + N_2 \qquad \text{(IV)}$$

The CN_2 radical should be stabilized by several resonance structures. If at least some of the nitrogen molecules formed in step (IV) were in the $A^3\Sigma$ state, this catalytic recombination could explain the chain characteristics of these flames since the $N_2(A^3\Sigma)$ molecule could initiate further reactions (step (I)). Two nitrogen atoms recombining into the lowest vibrational level of the $A^3\Sigma$ state provide 83 kcal, which could be used to excite the CN radical. Since the CN_2 radical would probably have a significant binding energy, and since it is possible to form $N_2(A^3\Sigma)$ molecules in vibrational levels other than the lowest, it is expected that any $CN(A^2\Pi)$ formed during such a cycle would have less than the full 83 kcal. The average amount of excitation energy of the CN molecules which are responsible for the P_2 emission peak is 56 kcal.

Since the emission curve for the CCl_4 flame (Fig. 2, solid line) is very similar to the P_2 peak in the HCN reaction (Fig. 3), it was thought that the same mechanism was responsible for both emissions. However, the addition of 1.2×10^{-5} mole/sec of hydrogen to a CCl_4 flame (CCl_4 flow 1.0×10^{-5}) did not strongly quench the CN emission. The emission from the 6th, 7th, and 8th vibrational levels of $A^2\Pi$ appeared to be reduced slightly, compared to the rest of the spectrum, but the effect was comparable with the experimental error. Thus it appears that the CN emission in the CCl_4 flame and the P_2 emission in the HCN reaction occur by different mechanisms, in spite of the similar spectral distributions.

The conclusions of this study can be summarized. (1) The second reactive species in active nitrogen, which is responsible for decomposing ammonia, initiates the reactions which cause the CN emission. (2) Chain reactions are involved in the CN emission. (3) There are at least three reactions producing electronically excited CN radicals, one yielding $B^2\Sigma$ state molecules and two yielding $A^2\Pi$ state molecules. (4) The CN emission from CCl_4, $HCCl_3$, and $HCBr_3$ flames cannot be accounted for by a reaction between active nitrogen and the products of the primary reactions.

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SOME NEW COMPONENTS OF ROYAL JELLY[1]

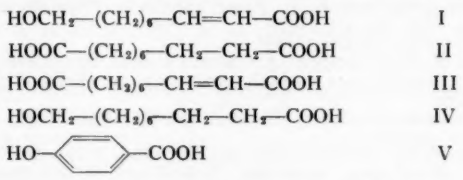
WILLIAM H. BROWN,[2] ETHEL E. FELAUER, AND ROBERT J. FREURE[3]

ABSTRACT

Two additional acids, 10-hydroxydecanoic and *p*-hydroxybenzoic, have been isolated from the ether-soluble fraction of royal jelly. A sterol, 24-methylenecholesterol, has been found in the ether-insoluble, methanol-soluble fraction of royal jelly.

Until recently, the only acid known to be present in royal jelly was 10-hydroxy-2-decenoic acid (I) isolated by Townsend and Lucas (1), and characterized by Butenandt and Rembold (2). In 1959 Brown and Freure (3) reported the isolation and identification of two additional acids, sebacic (II) and 2-decendioic (III).

By further investigation of the ether-soluble fraction of royal jelly we have isolated and identified two more acids, 10-hydroxydecanoic (IV) and *p*-hydroxybenzoic (V).



When the mixture of ether-soluble acids from royal jelly, after removal of the neutral compounds and phenols as previously described (3), is allowed to stand at room temperature for a week a layer of water forms in the bottom of the container and the oil gives a positive ester test. From the oil a neutral compound (probably a mixture of lactones) can be separated which on saponification gives 10-hydroxydecanoic acid (IV). Lactone formation is undoubtedly due to the catalytic effect of residual sulphuric acid which is present in the mixture of acids, A (3). Lactones of IV have been prepared and described by Stoll and Rouvé (4). Authentic 10-hydroxydecanoic acid was prepared from castor oil by a modification of a patented procedure (5). No previous report of the finding of IV as a naturally occurring acid could be found although higher hydroxy acids such as 14-hydroxypalmitic acid are present as esters in common beeswax (6). Subsequent to the submission of this communication Weaver and Law (7) reported the presence of IV in royal jelly.

Following chromatographic methods as previously described (3) we have also isolated *p*-hydroxybenzoic acid (V) from royal jelly. This acid is widely distributed in nature. Borner (8) isolated V by chromatographic means from the aqueous extracts of straw from grains such as rye, wheat, and barley. Jarczynski, Kiermerier, and Gossel (9) have shown that V is a natural ripening agent of cheese. Also, Davis (10) in studies using *E. coli* and *A. aerogenes* associated with their enzyme systems has demonstrated vitamin activity with V. The concentration of V in royal jelly is very small (30 p.p.m.).

Table I gives the concentrations and R_f values of the acids which have been isolated from royal jelly to date.

[1]*Manuscript received August 17, 1960.*
Contribution from the Department of Chemistry, Ontario Agricultural College, Guelph, Ontario.
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TABLE I

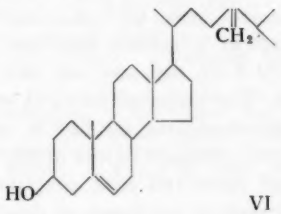
Concentrations and R_f values of acids present in royal jelly

Acid	Concn. in p.p.m. (basis lyophilized royal jelly)	R_f value*
I	70,000	0.79
II	200	0.44
III	300	0.39
IV	5,000	0.83
V	30	0.27

*Determined at 25° using a 7:3 ratio by volume of propanol and 28% aqueous ammonia solution as the developing solvent on Whatman No. 1 chromatography paper.

Methanol extraction of lyophilized royal jelly, previously extracted with ether, led to the isolation of a sterol, m.p. 139–141°, concentration 500 p.p.m.

The infrared absorption spectra of both the free sterol as well as its O-acetyl derivative are identical with those of authentic 24-methylenecholesterol (VI) and O-acetyl-24-methylenecholesterol, published by Barbier and Schindler (11). A mixture melting point between the O-acetyl derivatives was not depressed.



VI

Various melting points have been given for VI, depending upon the source from which it has been isolated. The reported melting points of the free sterol and the acetate derivative are listed in Table II.

TABLE II

Comparison of melting points of 24-methylenecholesterol (VI)

Source	Free sterol	Acetate derivative
From oysters (12)	142°	136°
From queen bees (13)	138–145°	131–136°
From worker bees (13)	138–140°	130–134°
Synthetic compound (14)	143°	135°
From royal jelly	139–141°	129–133°

The occurrence of VI in biological material has been reported twice. Barbier *et al.* (13) demonstrated the presence of VI in the neutral fraction of whole-bodied queen and worker bee extracts. Idler *et al.* (12) reported the isolation of VI from acetone extracts of the oyster (*Ostrea gigins*) and the clam (*Saxidomas giganteus*). Idler *et al.* (14) synthesized VI from 24-ketocholesterol and showed its identity with the natural product.

EXPERIMENTAL

All melting points reported by us have been corrected against reliable standards. Infrared data were determined by us on a Beckman IR5 instrument.

Isolation and Identification of 10-Hydroxydecanoic Acid (IV)

When 44 g of the mixture of ether-soluble acids, A (see Brown and Freure (3)), obtained from 390 g of lyophilized royal jelly, was allowed to stand at 25° for 1 week the oil did not crystallize but a second liquid phase appeared in the bottom of the flask. This liquid, 1.5 g, was removed and identified as water. The non-aqueous phase, which gave a positive test for sulphate ion, was processed again for the removal of neutral compounds as previously described (3). It yielded 1.85 g of a neutral colorless liquid, b.p. 270°; d_4^{20} 0.946; η_D^{20} 1.4472. The liquid gives a positive ester test.

Saponification of 1.5 g of the neutral liquid with 5% aqueous sodium hydroxide solution yielded 1.5 g of crude acid, m.p. 56–64°. One crystallization from water and two crystallizations from hexane (250 ml/g) gave 0.5 g of white needles melting at 71.5–72.5° both by itself and in admixture with authentic 10-hydroxydecanoic acid (IV).

Isolation and Identification of p-Hydroxybenzoic Acid (V)

The initial separation of V from the mixture of acids present in A (ether-soluble carboxylic acids. See Brown and Freure (3).) was carried out by chromatographing 0.30 g of A on 9×22.5 in. sheets of Whatman seed test paper as previously described (3). The area of paper, R_f 0.22–0.30, was cut out and extracted with propanol in a Soxhlet apparatus for 15 hours. The propanol extracts were combined, concentrated to a volume of 2 ml, and fractionated on four, 9×22.5 in. sheets of 3MM chromatography paper in order to remove residual amounts of the acids II and III. The area of paper, R_f 0.22–0.28, was cut out and extracted with propanol. The propanol extract was evaporated to dryness and the residue processed as described for the isolation of acids II and III (3). From 15 g of A, 0.014 g of crude V, m.p. 205–214°, was obtained. The crude V was washed with four 2-ml portions of cold chloroform (5°) to remove a sticky brown residue extracted from the chromatography paper. The resulting white residue, 0.011 g, had m.p. 211–215°. Crystallization from chloroform (55 ml/g) gave 0.0035 g of acid, m.p. 214–215°. Inspection of the infrared spectrum (KBr disk) of the sample suggested that it is *p*-hydroxybenzoic acid, a supposition which was confirmed by mixture melting point.

Blank determinations showed that V came from A and not from the possible breakdown of small amounts of lignin in the chromatography paper.

Isolation and Identification of 24-Methylenecholesterol (VI)

Ether-extracted, lyophilized royal jelly, 647 g, was placed in a Corning 3885 Soxhlet apparatus in 2-in. layers alternating with Pyrex glass fibers and extracted with absolute methanol for 2.5 hours. The dark orange extract was concentrated by distillation to 500 ml and cooled to 5°. White, plate-like crystals, which formed, were removed by filtration, weighed 0.278 g and had m.p. 122–135°. By concentrating the mother liquor and cooling, another 0.037 g of crystals, m.p. 125–137° was obtained. The crude product, 0.315 g, (500 p.p.m.) was crystallized twice from acetone (100 ml/g) including treatment with decolorizing charcoal. By this process there was obtained 0.029 g of white plates, m.p. 139–141°.

The compound gives a positive Liebermann–Burchard test and ferric chloride – sulphuric acid reaction (15) thus indicating that it is a sterol. The acetate, prepared according to the procedure of Barbier and Schindler (11), has m.p. 129–133°. A mixture melting point with authentic O-acetyl-24-methylenecholesterol was not depressed. Finally, the infrared spectra of the free sterol and the acetate are identical with those of authentic 24-methylenecholesterol and its acetate.

ACKNOWLEDGMENTS

Financial support for one of us (E.E.F.) was through a Public Health Research Grant. W. C. Hardesty Co. of Canada, Ltd. supplied generous amounts of 10-hydroxydecanoic acid. Professor G. F. Townsend, Department of Apiculture, O.A.C., Guelph, Ont., made available to us the lyophilized royal jelly used in the project. Dr. E. Reiner, Fisheries Research Board of Canada, Vancouver, B.C., kindly provided us with an authentic sample of 24-methylenecholesterol.

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LYCOPODIUM ALKALOIDS

PART IV. ALKALOIDS OF JAMAICAN LYCOPODIUM CLAVATUM LINN.[1,2]

R. H. BURNELL AND B. S. MOOTOO

ABSTRACT

Seven alkaloids have been isolated from *L. clavatum* Linn. collected in Jamaica, clavolonine $C_{16}H_{25}NO_2$, fawcettiine $C_{18}H_{29}NO_3$, base A (now named fawcettimine), lycopodine, dihydrolycopodine, and L.2 (acetyldihydrolycopodine). Fawcettimine is a secondary amine now shown to be $C_{16}H_{25}NO_2$. The isolation of deacetylfawcettiine $C_{16}H_{27}NO_2$ in quantity from a later extraction indicated that there was a seasonal variation in the alkaloid content of the plant. These bases differ from those obtained from *L. clavatum* of Canadian or European origin.

Lycopodium clavatum Linn. occurs in many areas of the world and at least two previous extractions are reported of the plant collected in Poland (1) and Canada (2). Since different bases were obtained from these previous extractions it seemed worth while to investigate material from a tropical source such as Jamaica. Club mosses do not grow in abundance in the tropics except at relatively high altitudes so the plant used for this work was collected in the Blue Mountains of Jamaica (ca. 5500 ft).

The extraction procedures follow closely the method used by us to extract *L. fawcettii* (3) and the yield of crude base amounted to 0.2% of the weight of dried plant when collected in April. Upon subjecting the crude alkaloid to a diagonal distribution between chloroform and buffer, three distinct fractions were obtained: (i) weak bases, (ii) a base of intermediate basicity, and (iii) strong bases. The latter afforded a crystalline base $C_{16}H_{25}NO_2$, m.p. 238°, which showed both hydroxyl and carbonyl absorption in the infrared and so was named clavolonine. The structure of the latter became apparent during work on fawcettiine (4).

The clavolonine mother liquors were acidified with perchloric acid and gave, in high yield, a perchlorate $C_{18}H_{29}NO_3.HClO_4$, m.p. 272–275°, which has been shown by the usual methods to be fawcettiine perchlorate, previously isolated from *L. fawcettii* (3, 5). Chromatography of the base regenerated from the mother liquors yielded more fawcettiine and clavolonine and another base, m.p. 168°, which proved to be dihydrolycopodine, $C_{16}H_{27}NO$.

The compound of intermediate basicity could not be crystallized but gave a methiodide, m.p. 240–242°, and a perchlorate, m.p. 225–226°, neither of which depressed the melting point of the corresponding derivative of base A, also obtained previously from *L. fawcettii*. Analyses of the free base, its hydrochloride, and perchlorate have prompted us to correct the molecular formula of base A to $C_{16}H_{25}NO_2$. We have named this compound fawcettimine since analysis of the methiodide, $C_{18}H_{30}NO_2I$ and the conversion of the base to an N-nitroso derivative (6) show it to be a secondary amine. Acetylation affords neutral N-acetylfawcettimine but this derivative has never been obtained crystalline. An infrared spectrum of the compound in carbon tetrachloride solution shows one carbonyl band at 1730 cm^{-1} whereas the methiodide shows two peaks at 1692 and 1730 cm^{-1}. The salts (perchlorate and hydrochloride) show only the 1690 cm^{-1} band in the carbonyl region.

[1]*Manuscript received November 7, 1960.*
Contribution from the Chemistry Department, University College of the West Indies, Jamaica.
[2]*Part III. Burnell and Taylor, Chem. & Ind. (London), 1239 (1960).*

The weak bases consisted of lycopodine, acetyldihydrolycopodine (Marion's L.2 (7, 8), and an extremely labile, feebly basic substance which has, as yet defied all attempts at characterization.

In a later extraction using plant material collected in the same locality, but in July (of the same year) the yield of crude base amounted to only a little over 0.1%. Clavolonine, fawcettiine, and lycopodine were obtained but fawcettimine, easily recognized by the green coloration it gives with the platinic chloride – potassium iodide spray used for chromatograms, was absent. The work-up of the strong bases from this extraction differed from that of the earlier work due to the presence of deacetylfawcettiine $C_{16}H_{27}NO_2$ in abundance. The latter was obtained in trace amounts from *L. fawcettii* (5) but it was felt at the time that the conditions used for the extraction could have hydrolyzed some of the fawcettiine present. Using a milder extraction such as employed in this case excludes this possibility.

EXPERIMENTAL

All infrared figures are for nujol mulls unless otherwise stated.

Crude Base (April)

Through the dried, ground plant (42 kg) was percolated 2% aqueous tartaric acid, which was then concentrated to about one-tenth of its volume and continuously extracted with chloroform. After basification with ammonia, the crude alkaloid (73.6 g) was obtained by further chloroform extraction. The crude base was distributed between chloroform and phosphate buffer, pH 6.98 in large separatory funnels in a diagonal manner to give 10 fractions. The buffer layers contained the strong bases (27 g) and the first two chloroform fractions the weak bases (25 g). The intermediate funnels contained only one base in abundance (14 g).

Clavolonine

The strong base (27 g) was dissolved in acetone and allowed to cool and the crystalline material filtered off (9.67 g). After recrystallization from acetone, the clavolonine was sublimed for analysis, m.p. 238°. Found: C, 72.9; H, 9.7; N, 5.1; O, 12.0; C—CH_3, 5.8. $C_{16}H_{25}NO_2$ requires: C, 73.0; H, 9.6; N, 5.3; O, 12.1; C—CH_3, 5.7.

Infrared peaks at 1693 cm^{-1} (C:O) and 3180 cm^{-1} (OH). A well-defined peak at 1412 cm^{-1} is indicative of the grouping —CH_2—CO—.

The methiodide was recrystallized from ethanol/acetone, m.p. 325–328° (decomp.). Found: C, 50.5; H, 6.6; N, 3.4; O, 8.2; I, 31.8; N—Me, 3.9. $C_{16}H_{25}NO_2 . CH_3I$ requires: C, 50.4; H, 7.0; N, 3.4; O, 79; I, 31.3; N—Me, 3.7.

Fawcettiine Perchlorate

The clavolonine mother liquors were made acidic with an acetone solution of perchloric acid and a little ether added. The perchlorate was filtered and washed with acetone/ether and dried (11.3 g). The salt, recrystallized from acetone melted at 272–275° and showed no depression on admixture with fawcettiine perchlorate obtained from *L. fawcettii* (3). The infrared traces were superimposable.

Dihydrolycopodine

The base regenerated from the fawcettiine perchlorate mother liquors (8.0 g) was chromatographed over alumina in benzene. The first benzene eluate containing base afforded a pale yellow oil (1.28 g) which was taken up in ethyl acetate and cooled. The

lustrous prisms which separated (440 mg) were recrystallized from acetone, m.p. 168°. A second crop (170 mg) was obtained on further standing. Found: C, 77.4; H, 11.2; N, 5.4. $C_{16}H_{27}NO$ requires: C, 77.1; H, 10.9; N, 5.6.

The infrared showed hydroxyl absorption at 3150 cm^{-1}, showed no bands in the carbonyl region, and was identical with that of a sample of dihydrolycopodine prepared from lycopodine (9). Mixed melting points of the bases or the methiodides prepared from them (m.p. 283–287°) showed no depression. The methiodide was recrystallized from ethanol/acetone. Found: C, 52.3; H, 7.8; N, 3.7; I, 32.0. $C_{16}H_{27}NO.CH_3I$ requires: C, 52.2; H, 7.7; N, 3.6; I, 32.4.

Base A (Fawcettimine)

The intermediate funnels from the distribution yielded 14 g of crude base which was partitioned again between chloroform and phosphate buffer, pH 6.69, in a multiple extractor. The strong and weak bases were combined with the respective fractions from the first distribution and the intermediate base (4.48 g) run over alumina in benzene. The base obtained (4.07 g) was shown to be homogeneous by paper chromatography and sublimed as a glass (130° at 2 mm). Found: C, 72.7; H, 9.3; N, 5.6; O, 12.7; C—Me, 4.7. $C_{16}H_{25}NO_2$ requires: C, 73.0; H, 9.6; N, 5.3; O, 12.2; C—Me, 5.7.

The infrared spectrum of the base in carbon tetrachloride showed a carbonyl peak at 1730 cm^{-1} but in nujol mulls of the salts of fawcettimine the carbonyl peak was at 1690 cm^{-1}.

The methiodide was prepared in acetone and recrystallized from ethanol/acetone, m.p. 240–242°. Found: C, 51.5; H, 7.2; N, 3.1; I, 29.6. $C_{18}H_{30}NO_2I$ requires: C, 51.6; H, 7.2; N, 3.3; I, 30.3.

The hydrochloride was recrystallized from ethanol/acetone, m.p. 235° (decomp.). Found: C, 63.8; H, 8.7; N, 4.7; O, 10.8; Cl, 11.6. $C_{16}H_{25}NO_2.HCl$ requires: C, 64.1; H, 8.7; N, 4.7; O, 10.7; Cl, 11.8.

The perchlorate was recrystallized from isopropanol/acetone, m.p. 225–226°. Found: C, 52.4; H, 7.0; O, 26.7; Cl, 9.9. $C_{16}H_{25}NO_2.HClO_4$ requires: C, 52.8; H, 7.2; O, 26.4; Cl, 9.7.

N-Nitroso-fawcettimine

Fawcettimine (170 mg) was dissolved in glacial acetic acid (1 ml) and water (3 ml) and cooled in ice. A cold solution of sodium nitrite (170 mg) in water (2 ml) was added dropwise with stirring. Crystals appeared on standing in the cold (106 mg), m.p. 137–138°. The derivative was recrystallized from ethanol/water and showed a peak in the ultraviolet at 235 mμ (ϵ: 7000) (6). Found: C, 65.1; H, 8.8; N, 9.4; O, 16.4. $C_{16}H_{24}N_2O_3$ requires: C, 65.7; H, 8.3; N, 9.6; O, 16.4.

Active hydrogen determinations in anisole and pyridine gave 0%.

Lycopodine

The weak base (25 g) was distributed diagonally between chloroform and acetate buffer, pH 5.59, to remove traces of stronger bases and neutral materials. A portion of this purer base (4.15 g) was run over alumina in benzene and the first benzene eluate (2.97 g) dissolved in 2 *N* acetic acid and saturated potassium nitrate solution added. The turbid solution deposited crystals of lycopodine nitrate (1.2 g) on cooling (10). The base regenerated from the nitrate melted over the range 116–118° and showed a carbonyl peak in the infrared at 1710 cm^{-1}.

The methiodide was recrystallized from ethanol/acetone, m.p. 322–323° (inserted at

280°). Found: C, 52.5; H, 7.5; N, 3.8; O, 4.1. $C_{16}H_{25}NO.CH_3I$ requires: C, 52.4; H, 7.3; N, 3.6; O, 4.1.

Obtaining the lycopodine via the nitrate does not afford an effective separation since more of the base remains in solution than crystallizes out, so the weak base (8.5 g) regenerated from the nitrate solution and remaining on the column was shaken between chloroform and acetate buffer, pH 4.27, for 20 transfers. The lycopodine (plus one other base) was mostly in tubes 6–14 (3.09 g) and was obtained by conversion to the perchlorate (2.11 g). Tubes 17–19 contained a small amount of clavolonine (120 mg).

Acetyldihydrolycopodine (L.2)

Solution infrared spectra of the fractions from the weak base distribution showed that tubes 6–14 contained, in addition to lycopodine, another base containing an O-acetyl grouping and no hydroxyl. Fraction 8 (0.58 g) was hydrolyzed in methanolic sodium hydroxide and the product chromatographed over alumina. Lycopodine (0.29 g) was obtained and the only other base was dihydrolycopodine, m.p. 168° (105 mg). Since the latter is far too strong a base to be present as such the acetylated base in the plant must be L.2 (acetyldihydrolycopodine).

The first tubes of the distribution contained an extremely labile base which has, as yet, resisted all attempts at purification or characterization.

Crude Base (July)

The dried, ground plant (22 kg) was extracted as described above affording the crude base (25.0 g), which was diagonally distributed in funnels as described above. Funnels 1 and 2 (weak base: 9.9 g) contained lycopodine and funnels 3–8 (7.9 g) afforded clavolonine (2.22 g) and fawcettiine perchlorate (2.45 g). No fawcettimine was found.

Deacetylfawcettiine

Funnel 10 from the distribution (2.44 g) solidified on evaporation of the solvent and afforded a crystalline base (1.18 g) m.p. 205–208° from acetone. A second crop (300 mg) of the same base was obtained on further concentration. A comparison of the infrared spectrum of this base with that of deacetylfawcettiine and a mixed melting point determination showed that the two bases were identical.

ACKNOWLEDGMENTS

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PRESSURE EFFECT AND MECHANISM IN ACID CATALYSIS

VII. HYDROLYSIS OF METHYL, ETHYL, AND *t*-BUTYL ACETATES[1]

A. R. OSBORN[2] AND E. WHALLEY

ABSTRACT

The effect of pressures up to 3 kbar on the rate of the acid-catalyzed hydrolysis of methyl, ethyl, and *t*-butyl acetates in dilute aqueous acid and of ethyl acetate in concentrated hydrochloric acid has been measured. The volume of activation for *t*-butyl acetate is zero within experimental error, showing that the mechanism is unimolecular. Those for methyl and ethyl acetates are near -9 cm^3 mole^{-1} in both dilute and concentrated acid. We deduce from this that the mechanism is the same in 9.2-*M* hydrochloric acid as in dilute acid, that the transition state is not highly polar, and that if the proton in the reactive protonated ester is on the carbonyl oxygen then the attacking water molecule adds, and if the proton is on the ether oxygen then the attacking water molecule substitutes.

1. INTRODUCTION

In spite of a large amount of experimental work, the hydrogen-ion-catalyzed hydrolysis of simple aliphatic esters is not well understood. There is little doubt, because, for example, of the relative rates in water and heavy water (1–3), that a pre-equilibrium proton transfer occurs, but the location of the proton in the reactive conjugate acid is not known. There is little doubt, mainly because of substituent effects (4, p. 770), that hydrolysis occurs by an attack of a water molecule on the conjugate acid, but it is not known whether the attack is an addition to a water molecule to form a stable intermediate or whether it is a simple substitution. It is, however, known that when ethyl benzoate is hydrolyzed by acid catalysis, the carbonyl oxygen of the unhydrolyzed ester exchanges with the oxygen of the solvent water (5, 6), but it is not known whether the hydrolysis and exchange follow similar or unrelated mechanisms. There are differing opinions (e.g. refs. 7–11) about whether the transition states that determine the rates have high dipole moments or not.

The effect of pressure on the rates of acid-catalyzed hydrolyses can provide useful information about the mechanisms (11–16), and a preliminary discussion of the hydrolysis of methyl acetate based on a few results reported by Bogojawlensky and Tammann (17) has already been given (11). In this paper we report measurements of the effect of pressures up to 3 kbar on the rates of hydrolysis of methyl and ethyl acetates in dilute hydrochloric acid.

Tal'vik and Pal'm (18) have argued that in concentrated acids ethyl acetate becomes essentially completely protonated, and that if allowance is made for the protonation, then the rate becomes nearly proportional to Hammett's acidity function h_0 rather than to the concentration of hydrogen ion. By using the Zucker–Hammett hypothesis (19, 20) they deduced that the slow step is

$$CH_3{-}C(=O)(H)(^+OC_2H_5) \rightarrow CH_3CO^+ + C_2H_5OH. \qquad [1]$$

An attempt to verify this mechanism in concentrated acids has been made by measuring the volume of activation in concentrated hydrochloric acid.

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[2]*N.R.C. Postdoctorate Fellow 1958–60.*

t-Butyl acetate hydrolyzes by acid catalysis (21–26) with alkyl–oxygen fission (26) and with an Arrhenius energy of 27.6 kcal mole^{-1} and an entropy of activation of 14.2 cal deg^{-1} mole^{-1} (22, 25) and it seems likely that the mechanism is unimolecular. The effect of pressure on the rate of hydrolysis in water has been measured to test this mechanism.

2. EXPERIMENTAL METHODS

The methyl and ethyl acetates used were of analytical grade and were not further purified. The *t*-butyl acetate was a commercial sample and it was distilled, the fraction b.p. 94–96.5° C being retained.

The high-pressure technique was similar to that used previously (12), except for the following modifications. In the original apparatus oil was pumped by hand into the pressure vessel to prevent loss of pressure when a sample was taken. We have added an air-driven oil pump to the apparatus, which automatically maintains the pressure constant when a sample is taken. The pump was modified from one made by the Sprague Engineering Co. by fitting it with a high-pressure end capable of generating a pressure of 4 kbar when 100 p.s.i.g. of compressed air is supplied to the low-pressure end.

In the original apparatus the reacting solution was in contact with an austenitic-stainless-steel capillary tube, which corrodes in strong acid. The sampling system was modified so that the solution is in contact with only glass, tantalum, Hastelloy B (Haynes Stellite Co.), and neoprene, all of which are unaffected for practical purposes by concentrated perchloric and hydrochloric acids up to at least 8 *M* near room temperature and will withstand more dilute acids up to at least 100° C. The new sampling system is shown in Fig. 1. The hypodermic syringe which is used as the reaction vessel is attached to the tantalum capillary sampling tube by means of the stainless-steel union and the small neoprene seal which isolates the reaction solution from the hydraulic oil. A small nut and lock nut at the upper end of the union allows the force on the neoprene seal to be adjusted. The union is held in place on the syringe by means of an o-ring which fits into a groove in the neck of the syringe. The two parts of the union are held together by means of a bayonet-and-socket device similar to that used on some lamp bulbs. The sampling valve is made of Hastelloy B, and the connection between the valve and the tantalum tube is shown in the upper part of Fig. 1. A metal–metal seal isolates the hydraulic oil from the atmosphere, and an o-ring seal isolates the oil from the sample. This connection can be easily dismantled when necessary. The stem of the valve is also made of Hastelloy B. The outlet of the valve is connected to a water-jacketed tantalum capillary tube.

The hydrolyses were followed by titration with standard alkali of the acid produced during the hydrolysis, using phenolphthalein as indicator.

3. RESULTS

The reactions follow first-order kinetics and the first-order constants were obtained graphically in the usual way. The accuracy was about 2%. For reactions in dilute aqueous acids, the second-order constants k_a were obtained by dividing the first-order constants by the concentration of acid at the temperature and pressure of the experiment, assuming that the compression and thermal expansion of the solution are the same as that of pure water (27). For reactions in concentrated acids the first-order constants k_1 are reported because the compressibility of concentrated hydrochloric acid has not been measured. The rate constants are given in Tables I and II and are shown graphically in Fig. 2.

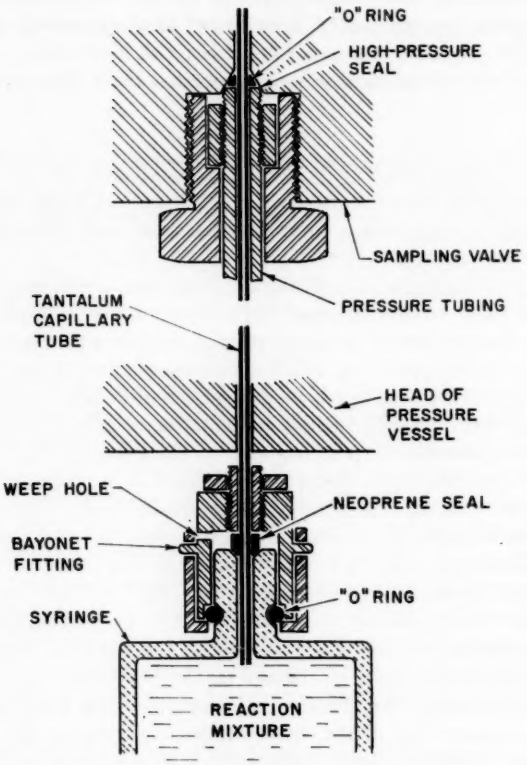


FIG. 1. Sampling system for high-pressure apparatus.

TABLE I

Acid-catalyzed hydrolysis of methyl acetate in aqueous solution at 35.00° C with 0.1-*M* HCl

p/bar	$10^6 k_a$/l. mole^{-1} sec^{-1} in water
1	2.82
250	3.09
507	3.27
507	3.29
1000	3.98
1500	4.64
2000	5.24
2500	6.06
3000	6.90

For comparison, the second-order rate constant for the hydrolysis of methyl acetate at 35.00° C, is reported to be 277×10^{-6} l. mole^{-1} sec^{-1} in 0.1-*M* hydrochloric acid (28). For ethyl acetate interpolation of Tommila and Hella's (29) results gives 269×10^{-6} l. mole^{-1}

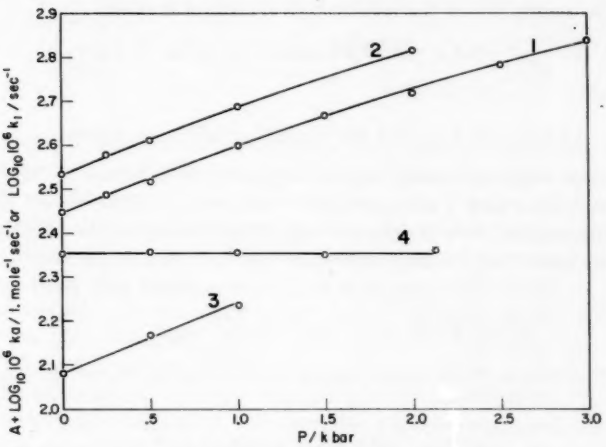


FIG. 2. Hydrolysis of acetates catalyzed by hydrochloric acid. Curve 1, k_a for methyl acetate in 0.1-*M* aq. HCl at 35.00° C, $A = 0$; curve 2, k_a for ethyl acetate in 0.1-*M* aq. HCl at 35.00° C, $A = 0.1$; curve 3, k_1 for ethyl acetate in 9.21-*M* aq. HCl at 0.00° C, $A = 0$; curve 4, k_a for *t*-butyl acetate in 0.01-*M* aq. HCl at 60.00° C, $A = -1.9$.

TABLE II

Acid-catalyzed hydrolysis of ethyl acetate in aqueous solution

p/bar	$10^6\ k_a$/l. mole^{-1} sec^{-1}	p/bar	$c°_{HCl}/M$	$10^6\ k_1$/sec^{-1}
1	273	1	2.35	21.7
250	301	1	4.63	51.5
500	325	1	7.18	98.3
1000	387	1	9.21	120
2000	520	1	9.21	120
		500	9.21	147
		1000	9.21	172
35.00° C, 0.1-*M* HCl		0.00° C, $c°_{HCl}$ = concn. of HCl at room temperature and pressure		

TABLE III

Acid-catalyzed hydrolysis of *t*-butyl acetate in water at 60.00° C with 0.01-*M* HCl

p/bar	$10^3\ k_a$/l. mole^{-1} sec^{-1}
1	17.9
500	18.0
1000	18.0
1500	17.8
2000	18.2

sec^{-1} for k_a at 35.00° C in water. For *t*-butyl acetate interpolation of Palomaa *et al.*'s results (22) gives 17.6×10^{-3} l. mole^{-1} sec^{-1}. The agreement is satisfactory.

The volumes of activation ΔV^* were obtained graphically (Fig. 2) using the usual relations

$$\partial \ln k_a/\partial p = -\Delta V^*/RT \text{ or } \partial \ln k_1/\partial p = -\Delta V^*/RT + \kappa,$$

where κ is the compressibility of the solution. For methyl acetate,

$$\Delta V^{\ddagger} = -9.1 \pm \sim 0.7 \text{ cm}^3 \text{ mole}^{-1} \text{ at } 35^\circ \text{ C in water.}$$

For ethyl acetate

$$\Delta V^{\ddagger} = -9.3 \pm \sim 0.7 \text{ cm}^3 \text{ mole}^{-1} \text{ at } 35^\circ \text{ C in water.}$$

These compare with -8.3 cm^3 mole^{-1} for methyl acetate in water at 30.5° C calculated (11) from Bogojawlensky and Tammann's (17) rate constants at 1 and 500 atm. Rothmund's (30) measurements, which were the second published on the effect of pressure on reaction rates in solution, yield -10.1 cm^3 mole^{-1} for methyl acetate and -8.4 cm^3 mole^{-1} for ethyl acetate at 14° C. The rates in 9.21-*M* hydrochloric acid yield graphically

$$RT\partial \ln k_1/\partial p = 9.1 \pm \sim 1.0 \text{ cm}^3 \text{ mole}^{-1}.$$

If the compressibility of 9.21-*M* hydrochloric acid is taken to be the same as that of pure water then

$$\Delta V^{\ddagger} = -7.9 \pm \sim 1.3 \text{ cm}^3 \text{ mole}^{-1},$$

where the error has been increased to allow for the uncertainty of the compressibility of the acid solution.

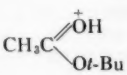
For *t*-butyl acetate

$$\Delta V^{\ddagger} = 0.0 \pm \sim 1.0 \text{ cm}^3 \text{ mole}^{-1} \text{ at } 60^\circ \text{ C.}$$

4. DISCUSSION

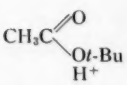
1. t-Butyl Acetate

The hydrolysis of *t*-butyl acetate is the simplest, and it is discussed first. The volume of activation of $0.0 \pm \sim 1.0$ cm^3 mole^{-1} is clearly consistent with either of the likely (see Section 1) unimolecular slow steps



$$\rightarrow CH_3CO_2H + t\text{-}Bu^+,$$

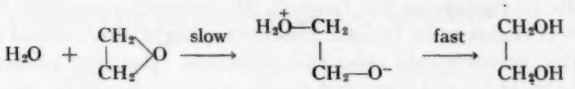
or



$$\rightarrow CH_3CO_2H + t\text{-}Bu^+.$$

2. Polarity of the Transition States for Methyl and Ethyl Acetates

The volumes of activation for the acid-catalyzed hydrolysis of methyl and ethyl acetates in dilute aqueous acid at 35° C are about -9.1 and -9.3 cm^3 mole^{-1}. As was pointed out earlier (11) these are approximately the values expected for a bimolecular reaction of a water molecule with a positively charged substrate if no major change in electrostriction due to changes in electrostatic moments occurs. In confirmation of this they are within about 1.5 cm^3 mole^{-1} of the volumes of activation for the acid-catalyzed hydrolysis of several aliphatic epoxides (13) and diethyl ether (14), for which the transition states are unlikely to have high dipole moments. They are much higher than the volume of activation for the spontaneous hydrolysis of ethylene oxide, which is -15.9 to -19.9 cm^3 mole^{-1} at 25 to 70° C (31), whose mechanism is



where the transition state is almost certainly very polar. In addition, all the volumes of activation so far measured of the reactions of small negative ions such as hydroxide and ethoxide with small neutral molecules lie in the range -5 to -10 cm^3 mole^{-1} (31). We conclude therefore that the transition state of the acid-catalyzed hydrolysis of simple esters is not highly polar and in particular that the carbonyl group is not appreciably ionized in the transition state.

Tal'vik and Pal'm's (18) mechanism, equation [1], should give a positive or zero volume of activation like *t*-butyl acetate, sucrose (11), formals and acetals (12), and α-D-glucose-1-phosphate (16), and it can be eliminated by these results, at least in dilute acid. The volume of activation of ethyl acetate appears to be little different in 9.21-*M* hydrochloric acid, in which the acidity function $-H_0$ is about 3.5, from that in dilute acid. In sulphuric acid the rate is reported (18) to be independent of acid concentration for $-H_0 > 2$ and it was deduced that the ester is essentially completely protonated. It is likely from the small change in the volume of activation that no change of mechanism has occurred in concentrated acid.

It has been argued (8–10) that the effect of solvent (7, 10, 29, 32, 33, 34) on the rates of these hydrolyses shows the transition states to be very highly polar, having dipole moments of 26.1 D. It has also been argued (e.g. 7, 34) that the same results show the transition state to be not highly polar. The solvent effects are small, the second-order rate constants typically decreasing by a few tens per cent in the range zero to 50% v/v acetone in water, and by similar amounts in other aqueous solvents. These are very much smaller effects than occur in reactions between two neutral molecules in which a highly polar transition state is formed, for which there is a large decrease in the rate constant as the dielectric constant of the solvent decreases (4, p. 345 *et seq.*). They are similar to, and in fact less than, the solvent effects for the acid-catalyzed hydrolysis of ethylene oxide in methanol–water mixtures (35) and in ethanol–water mixtures (36) for which the transition states are thought not to be highly polar. The solvent effects therefore confirm that the transition states for the acid-catalyzed hydrolysis of simple esters and of ethylene oxide have similar polarity.

Theories (8) that invoke highly polar transition states for the hydrolysis of esters suffer also from the following defects.

(i) They predict a large variation in entropy and enthalpy of activation with varying solvent, whereas the entropy of activation for the acid-catalyzed hydrolysis of methyl acetate differs by less than 10% between solvent water and solvent 50% v/v acetone–water (29).

(ii) They require radii of the transition states that seem too large. Thus, for the hydrolysis of ethyl acetate in dioxane–water mixtures the radius of the transition state is given (8) as 4.6 Å, which corresponds to a volume, calculated as $(4/3)\pi Na^3$ where a is the radius and adding one-third for the unoccupied volume, of 328 cm^3 mole^{-1}, and in methanol–water mixtures a radius of 5.7 Å is required (10), which corresponds to 620 cm^3 mole^{-1}.

Two other objections to the details of Laidler and Landskroener's mechanism are as follows. The hydrolyses are a good deal faster in heavy water than in ordinary water,

which is difficult to explain on the basis of the assumed transition states because they require two O—H bonds to be broken. However, a slight alteration of two O—H distances in their transition states would meet this objection. Also, the assumed transition state incorporates H_3O^+, H_2O, and ester and so the reaction should be third-order. The third-order rate constants *increase* with increasing concentration of organic solvent (7, 34).

We conclude therefore that the transition state for the hydrolysis of simple aliphatic esters is not very polar.

3. Location of the Proton

The conclusions that we can draw about the location of the proton in the reactive conjugate acid of the ester are intimately connected with the problem of whether the attack by water is a substitution or an addition. The exchange of ^{18}O between hydrolyzing ethyl benzoate and the solvent (5, 6) show that water can add reversibly to the ester, and though it is not unlikely that the adduct is an intermediate in the hydrolysis, this has not yet been conclusively demonstrated. It has been shown (11) that if the transition state is not very polar, then either the proton is on the carbonyl oxygen and the water molecule adds or the proton is on the ether oxygen and the water molecule substitutes. The demonstration given above that the transition state is not very polar confirms this conclusion.

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PRESSURE EFFECT AND MECHANISM IN ACID CATALYSIS

VIII. HYDROLYSIS OF ACETAMIDE AND BENZAMIDE[1]

A. R. OSBORN,[2] T. C-W. MAK, AND E. WHALLEY

ABSTRACT

The effect of pressures up to 3 kbar on the rate of the acid-catalyzed hydrolysis of acetamide and benzamide in both dilute and concentrated perchloric acid has been measured. The volumes of activation in dilute acid are consistent with a transition state that is not highly polar. It follows from this that if the attacking water molecule adds to the amidium ion then the reactive amidium ion is the O-protonated form, and if the attacking water molecule substitutes then the reactive amidium ion is the N-protonated form.

The volume of activation for acetamide in concentrated acid provides no additional information about the mechanism. That for benzamide in concentrated acid is tentatively interpreted as favoring the O-protonated benzamidium ion as the reactive ion.

1. INTRODUCTION

The acid-catalyzed hydrolysis of amides is in many ways similar to the acid-catalyzed hydrolysis of carboxylic esters, and many of the questions that require solution are similar to those for esters (1). It seems certain that the mechanism is

$$RCONH_2 + H_3O^+ \rightleftharpoons RCONH_2.H^+ + H_2O, \qquad \text{equilibrium} \qquad [1]$$

$$RCONH_2.H^+ + H_2O \rightarrow \text{products}. \qquad \text{slow} \qquad [2]$$

The relative rates of hydrolysis of acetamide in dilute acid in water and heavy water (2) show that a pre-equilibrium proton transfer occurs, and mechanisms that omit this (3, 4, 5) are almost certainly wrong. The location of the proton is unknown. Substituent effects (6, 7) indicate that a bimolecular attack of water probably occurs, but it is not known whether the attack is by substitution or by addition. Hydrolyzing benzamide does not exchange its oxygen with the solvent (8, 9) but this does not, of course, prove that an intermediate molecule is not formed, but only that if one is formed then it decomposes to products much faster than it reverts to reactants. Useful information about the mechanism of hydrolysis of simple esters has been obtained by measuring the effect of pressure on the rate (1), and so the effect of pressure on the rate of hydrolysis of acetamide and benzamide has been measured.

It is well known that the rates of hydrolysis go through a maximum as the concentration of acid is increased, and there is no doubt that the amide becomes appreciably protonated in concentrated acids. So that something could be learned about the volume change of the proton-transfer reaction [1] and the volume of activation of the slow step [2], the effect of pressure on the rates in concentrated acid has also been measured.

2. EXPERIMENTAL METHODS

Reagent-grade acetamide was recrystallized from approximately 1:10 ether–ethanol (10). Eastman-Kodak benzamide was recrystallized from water to constant melting point.

The high-pressure technique was as described in previous papers (1). About five samples were taken for analysis during a run. The reaction mixtures were made from perchloric acid and amide solutions of known concentrations.

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[2]*N.R.C. Postdoctorate Fellow 1958–60.*

The hydrolysis of acetamide was followed by analyzing for the acetic acid produced. About 5 ml of reaction solution (initially either 0.2992 *M* in perchloric acid and 0.1980 *M* in acetamide or 3.99 *M* in perchloric acid and 0.0990 *M* in acetamide) were withdrawn and 3.00 ml pipetted into a 30-ml weighing bottle. One drop of thymol blue indicator was added and the solution was titrated with 2-*N* sodium hydroxide solution until the color of the indicator just changed from red to yellow (pH approximately 2.8–3.0). Six milliliters of methanol was added and the solution titrated potentiometrically with 0.1-*N* barium hydroxide solution, using a Beckman Model-G pH meter with a Beckman No. 40463 sleeve-type calomel electrode as reference. The first inflection, at about pH 4, corresponded to neutralization of the perchloric acid, and the second, at about pH 7.3, to neutralization of the acetic acid. The methanol was added to sharpen the first inflection. Both inflections were easily located to ±0.01 or 0.02 ml. A typical titration required 2 ml, so the error is about 1 to 2%.

The same method could not be used for benzamide because if its concentration was much greater than about 0.02 *M* benzoic acid precipitated and it may clog the high-pressure apparatus, and 0.02 *M* is too low a concentration for this method. The ammonia produced was therefore determined by the formol titration method (11, 12). About 5 ml of reaction mixture (initially about either 1 *M* or 4.08 *M* in perchloric acid and 0.02 *M* in benzamide) was withdrawn, weighed accurately, and neutralized approximately with 1-*N* sodium hydroxide solution. It was adjusted to pH 6.6 with 0.1-*N* barium hydroxide solution, using the pH meter described above. A 5-ml blank solution, identical with the above solution except that it contained no benzamide, was treated similarly. Five milliliters of formalin solution (50 ml of Merck formaldehyde (37.0–37.3%) plus 0.5 ml of phenolphthalein indicator (0.5% in 50% ethanol) neutralized to faint pink color with *N* sodium hydroxide) were added to each of the two solutions. The pH of the blank was determined, the sample titrated to the same pH with 0.1-*N* barium hydroxide, and the pH of the blank measured again. If the pH of the blank had changed, alkali was added to either sample or blank and the appropriate allowance made. The alkali was run in from a precision-bore capillary tube with a meter stick attached, so that the amount added was measured to ±0.005 ml. The titer gave the equivalent amount of acid liberated by the reaction of ammonia and formaldehyde to form hexamethylenetetramine. To obtain reproducible results the electrode was flushed frequently with saturated potassium chloride solution.

3. RESULTS

1. Acetamide

For the hydrolysis in dilute acid the second-order rate law is appropriate. The integrated form

$$k_a t = \frac{1}{a-b} \ln \frac{b(a-x)}{a(b-x)},$$

where a and b are the initial concentrations of perchloric acid and acetamide, x is the amount of each reacted per unit volume at time t, and k_a is the second-order rate constant, can be rewritten

$$\log_{10} \frac{a-x}{b-x} = \frac{a-b}{2.303} k_a(t-t_0) - \log_{10} \frac{a-x_0}{b-x_0},$$

where x_0 is the amount reacted per unit volume at any arbitrary starting time t_0. A plot of $\log_{10}[(a-x)/(b-x)]$ against t gives therefore a straight line of slope $k_a(a-b)/2.303$. The appropriate corrections for compression (13) and thermal expansion of the solution were made. The second-order rate constants are given in Table I.

TABLE I

Acid-catalyzed hydrolysis of acetamide at 55.00° C

p/bar	$10^6\,k_a$/l. mole^{-1} sec^{-1} (initially 0.2992-M $HClO_4$ 0.1980-M acetamide)	$10^6\,k_1$/sec^{-1} (3.99-M $HClO_4$ 0.0990-M acetamide)
1	195	109
500	228	130
1000	268	164
1500	332	205
1500	333	
2000	387	248

The volume of activation of the reaction

$$RCONH_2.H^+ + H_2O \rightarrow \text{products}$$

can, at least in principle, be obtained from rates in concentrated acid because in these solutions the amide becomes largely protonated. There is, however, a difficulty, that the rate does not become of zero order in acid, but the first-order (in amide) rate constant passes through a maximum at concentrations of acid that are dependent upon the acid and amide, but are often around 2 to 4 M. What allowance to make in the rate constant for the effect of pressure on the concentration is not entirely clear, and we have preferred to reduce the effect of this uncertainty by using acid concentrations not far above those corresponding to the maximum rate. This gain in using a low concentration of acid is partly offset by the lower degree of protonation of the amide, and the concentration chosen, which was 4 M, was a compromise.

For the hydrolysis in a solution initially 3.99 M in perchloric acid the first-order rate law is appropriate, and the first-order constants were obtained graphically in the usual manner; they are given in Table I. They were not converted to second-order constants because, as mentioned above, the reactions are not second-order in concentrated acid.

By interpolating Rabinovitch and Winkler's (14) results at atmospheric pressure we find that $10^6\,k_a$/liter mole^{-1} sec^{-1} in 1-M hydrochloric acid is 186 compared with our 195 in 0.3-M perchloric acid, and that $10^6\,k_1$/sec^{-1} in 4-M hydrochloric acid is 133 compared with our 109 in 4-M perchloric acid. The agreement is satisfactory considering that in concentrated acids exact agreement is not expected.

2. Benzamide

The concentration of benzamide was always low enough that the first-order law was followed. The rate constants are accurate to about 5%. The rate constants in 1-M perchloric acid were converted to second-order constants k_a in the usual way and they are given in Table II.

A few kinetic runs were made to determine approximately the acid concentration that gives the maximum rate of hydrolysis. The first-order constants are given in Table III. The high-pressure rate constants were determined in 4.08-M perchloric acid solution. Because the rate is not first order in acid in concentrated acid the first-order constants are listed in Table II.

TABLE II

Acid-catalyzed hydrolysis of benzamide at 80.00° C

p/bar	$10^6\ k_a$/l. mole^{-1} sec^{-1} (initially M $HClO_4$ 0.02-M amide)	$10^6\ k_1$/sec^{-1} (4-M $HClO_4$ 0.02-M amide)
1	60.4	99.4
500	73.3	129.2
	71.7	
1000	87.6	171
	87.6	
1500	107.7	228
	98.9	
2000	112.4	339
	109.9	
	114.6	

TABLE III

Hydrolysis of benzamide in different concentrations of acid at 80.00° C and atmospheric pressure (Initial concentration of benzamide was 0.02-M)

c_{HClO_4}/M	$10^6\ k_1$/sec^{-1}
1.02	60
2.04	104
2.86	114
4.08	99
4.08	99*

*Reaction carried out in pressure vessel.

For comparison, we find by interpolating Rabinovitch and Winkler's (14) results that in M hydrochloric acid $10^6\ k_1$/sec^{-1} is 59 compared with our 60.4 in M perchloric acid, and in 4-M hydrochloric acid $10^6\ k_1$/sec^{-1} is 219 compared with our 99 sec^{-1} in 4.08-M perchloric acid. The cause of the difference in concentrated acid is not immediately obvious.

3. Volumes of Activation

The rate constants as a function of pressure are shown in Fig. 1. The volumes of activation were obtained graphically from the slope of the graph of log k vs. p at zero pressure from the appropriate formula

$$\frac{\partial \ln k_a}{\partial p} = -\frac{\Delta V^{\ddagger}}{RT}, \quad \text{or} \quad \frac{\partial \ln k_1}{\partial p} = -\frac{\Delta V^{\ddagger}}{RT}.$$

The volumes of activation are given in Table IV.

TABLE IV

Volumes of activation for the acid-catalyzed hydrolysis of acetamide and benzamide

Amide	Concn. of $HClO_4/M$	Temp./ °C	$\Delta V^{\ddagger}$ cm^3 mole^{-1}
Acetamide	0.299	55	$-9.4\pm\sim0.5$
Acetamide	3.99	55	$-11.3\pm\sim0.8$
Benzamide	1.00	80	$-12.1\pm\sim1.0$
Benzamide	4.08	80	$-16.0\pm\sim0.7$

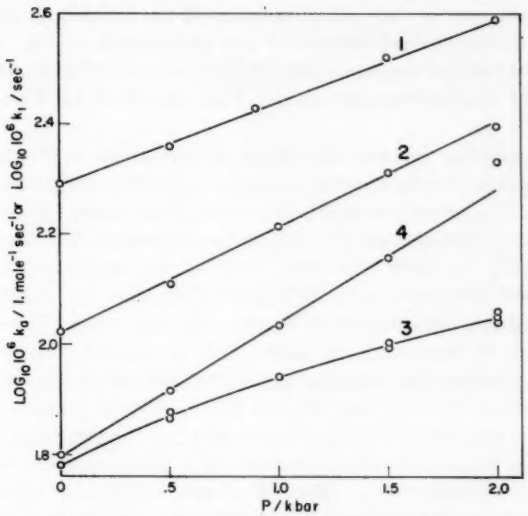


FIG. 1. Acid-catalyzed hydrolysis of acetamide and benzamide. Curve 1, k_a for acetamide at 35.00° C in 0.3-*M* $HClO_4$ and 0.2-*M* acetamide. Curve 2, k_1 for acetamide at 35.00° C in 4-*M* $HClO_4$ and 0.1-*M* acetamide. Curve 3, k_a for benzamide at 80.00° C in *M* $HClO_4$ and 0.02-*M* benzamide. Curve 4, k_1 for benzamide at 80.00° C in 4-*M* $HClO_4$ and 0.02-*M* benzamide, displaced downwards one unit for clarity.

4. Volume Changes of the Proton-Transfer Reactions

We assume for want of anything better that the volume change of reaction [1] and the volume of activation of reaction [2] are the same in dilute and in 4-*M* perchloric acid and that the pK_a's are independent of temperature (see, for example, ref. 7). There is some disagreement about the value of pK_a for the acetamidium ion (15, 16, 17, 18). The smallest pK_a, −1.40 (18), indicates that about two thirds of the amide is protonated in 4-*M* perchloric acid and the others indicate that more than this is protonated. It follows therefore that the volume change of the proton-transfer reaction (1) is $3.8 \pm \sim 2.5$ cm^3 mole^{-1} for acetamide.

The value of pK_a for the benzamidium ion has recently been revised from −1.85 (18) to −2.16 (19) and so it seems that benzamide is only about one-quarter protonated in 4-*M* perchloric acid, instead of about one-third as it seemed when this work was done. The volume change for the proton-transfer reaction [1] for benzamide appears to be therefore about $16 \pm \sim 8$ cm^3 mole^{-1}. These values are unfortunately rather rough, but they do seem to be significant.

4. DISCUSSION

1. Polarity of the Transition State

If the dipole moment of the transition state is much bigger than that of the amide, then there will be an increase in the electrostriction of the solvent when the transition state is formed (see, for example, preceding paper). The object of this section is to determine whether this occurs. Only the reaction

$$H_3O^+ + RCONH_2 \rightarrow \text{transition state}$$

need be considered for the present because in dilute acid, where the amide is only slightly protonated, the volume of activation is the difference between the partial volume of the

transition state and the sum of the partial volumes of the amide and the hydronium ion, and is independent of the partial volume of the protonated amide. This is important because the partial volume of the transition state can be discussed without reference to the partial volume of the protonated amide. This last shall be discussed in the next section.

The volume of activation for the hydrolysis of acetamide in dilute acids is $-9.4\pm\sim 0.5$ cm^3 mole^{-1}, which is almost identical with the volumes of activation for the hydrolysis of ethylene oxide (20), propylene oxide (20), isobutylene oxide (20), diethyl ether (21), methyl acetate (1), and ethyl acetate (1). The same arguments as those used in discussing the hydrolysis of esters (1) show that very little of the volume of activation can be accounted to increased electrostriction. It follows then that the dipole moment of the transition state is probably little different from that of the amide. The volume of activation for the hydrolysis of benzamide in dilute acid is somewhat lower, $-12.1\pm\sim 1.0$ cm^3 mole^{-1}, and it is possible that about 2 to 4 cm^3 mole^{-1} of this may be due to slightly increased electrostriction. It may also be due to the phenyl group's having a greater steric hindrance to the approach of a water molecule than the methyl group. We conclude therefore that no large separation of charges occurs in the transition states.

This conclusion is confirmed by the effect of solvent on the rate of the acid-catalyzed hydrolysis of acetamide in ethanol–water mixtures (4). This is very similar to that of the acid-catalyzed hydrolysis of ethylene oxide in the same solvent (22), confirming the conclusions drawn from the close similarity of the volumes of activation. Laidler and Landskroener (4) and Laidler (5) have argued that this solvent effect implies that the transition state for the hydrolysis of acetamide has a dipole moment of 18.4 D, but this is probably wrong. In addition, the detailed mechanism proposed by Laidler and Landskroener (4) requires two OH bonds to be broken in the transition state, and it is difficult to see how this can be reconciled with the observed faster rate in dilute acid in heavy water than in ordinary water. It seems certain that this demands a pre-equilibrium proton transfer to the amide. However, this can be accommodated in Laidler and Landskroener's transition states by altering two O—H distances.

2. Location of the Proton in the Reactive Amidium Ion

There has been much controversy about whether the extra proton in amidium ions is on the oxygen or the nitrogen atom. Proton resonance spectra (23, 24) appear to show conclusively that acetamide, N-methylacetamide, and N,N-dimethylacetamide are mainly O-protonated. There is no certain information about benzamide. The O-protonated form need not, of course, be the more reactive form.

The conclusions that we can draw about the location of the proton in the reactive form are intimately connected with the question whether the attack of water on the amidium ion is by addition to form a molecule followed by rapid elimination of NH_3 (or NH_2^-) or by direct substitution with simultaneous elimination of NH_3 (or NH_2^-), about which there is no direct evidence. Approximate representations of the transition states for the various mechanisms are given in Fig. 2. The only ones in which a great separation of charges has not occurred, and which therefore are consistent with the evidence discussed in the preceding section are substitution of the N-protonated form or addition to the O-protonated form.

3. Volume Change of the Proton-Transfer Reaction [1]

The evidence that will be discussed in this section is a good deal more uncertain than that discussed in preceding sections.

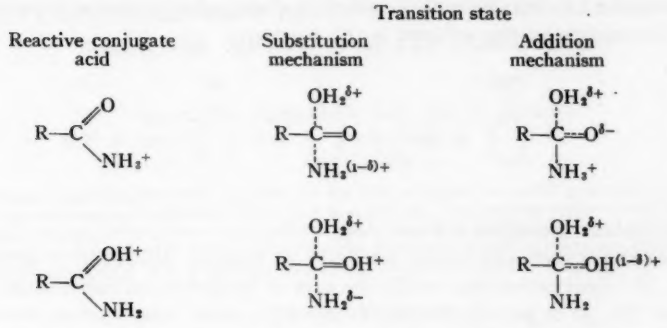
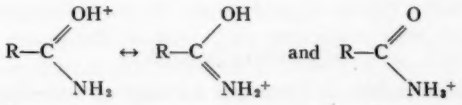


FIG. 2. Transition states for the acid-catalyzed hydrolysis of amides.

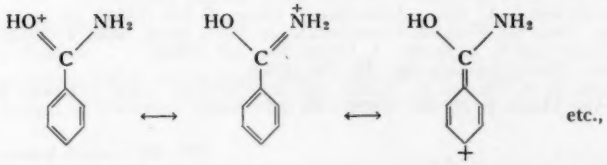
The two most likely amidium ions are



(the double-headed arrow indicates resonating forms). There will probably be an increase in volume when the O-protonated amidium ion is formed from amide and hydronium ion, because the positive charge becomes spread between the oxygen and the nitrogen atoms and this dispersion will decrease the electrostriction of the solvent. A similar, probably more complete, sharing of charge occurs when a proton is transferred from a carboxylic acid to a hydroxyl ion. The volume changes for this reaction for various acids, calculated from the data in the references quoted and the volume of ionization of water of -23.4 cm^3 mole^{-1} (25) are as follows: formic, 15.4 (26); acetic, 10.9 (26); propionic, 9.7 (26); *n*-butyric, 9.7 (26); fumaric, 11.0 (27); benzoic, 12.8 (28); *o*-hydroxybenzoic, 16.2 (28) cm^3 mole^{-1}. The increase of volume for the O-protonation of acetamide may not be so great as these because the sharing of the charge between the oxygen and the nitrogen atoms may not be equal. When the N-protonated amidium ion is formed, little dispersion of charge will occur, and so little change of volume is expected (29).

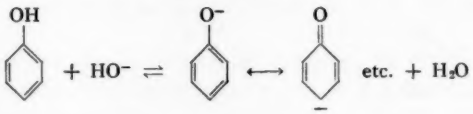
The volume of protonation of acetamide, $3.8\pm\sim 2.5$ cm^3 mole^{-1}, appears to be consistent with either of these protonations, and it does not allow us to choose between them.

The volume change for the protonation of benzamide, $16\pm\sim 8$ cm^3 mole^{-1}, appears to be too big for N-protonation. It is, however, quite consistent with O-protonation because in the O-protonated ion, which has the following main resonating forms



the charge is probably shared not only by the oxygen and the nitrogen atoms, but also

by the benzene ring. An example of the effect of dispersal of a charge into a benzene ring on the electrostriction of the solvent is the reaction



for which the volume change is 6.6 cm^3 $mole^{-1}$ (26).

This conclusion is certainly rather tentative at present. However, it does agree with Leisten's (7, 30) observation that while the rate of hydrolysis of substituted benzamides in dilute acid (31, 3) is practically uninfluenced by polar substituents, in concentrated acid, where the rate measured is largely the rate of reaction of the amidium ion with water, there are quite strong polar effects, electron-releasing substituents decreasing the rate and electron-withdrawing substituents increasing the rate. These are the effects that are expected if the attack of water results in the withdrawal of positive charge from the benzene ring.

ACKNOWLEDGMENTS

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SPECTROPHOTOMETRIC DETERMINATION OF ALUMINUM IN URANIUM METAL AND ITS COMPOUNDS[1]

ALLAN W. ASHBROOK AND G. M. RITCEY

ABSTRACT

A rapid spectrophotometric method for the determination of small amounts of aluminum in uranium metal and uranium compounds is described. The method makes use of the yellow-colored complex formed between aluminum and 8-hydroxyquinoline (oxine) at pH 9.5–10; the complex is extracted with carbon tetrachloride. Elements which interfere are extracted with 8-hydroxyquinaldine. The method is suitable for the determination of up to 0.1% aluminum.

INTRODUCTION

Aluminum is found in trace amounts in uranium metal and its compounds. Specifications limit its amount to 30 p.p.m. in uranium metal; consequently a sensitive and accurate method was required to determine this amount.

Aluminum has been determined by Horton and Thompson (1) after separation of most of the interfering elements by ion-exchange. Aurine tricarboxylic acid (Aluminon) was used as the chromophoric reagent. This procedure gave erratic results in this laboratory, and also suffered from non-uniformity of the reagent. Of four samples of Aluminon used, each gave a different calibration curve for aluminum.

Methods employing mercury cathode electrolysis for the removal of interfering elements are time consuming. Of other reagents available, oxine showed the best sensitivity (2). The use of this reagent has produced a simple and sensitive method for the determination of aluminum in uranium metal and its compounds. With slight modifications, it has also been used for the determination of small amounts of aluminum in uranium-containing steels.

EXPERIMENTAL

Apparatus and Solutions

The spectrophotometer used in these experiments was a Beckman Model B, with matched 5-cm glass cells.

A standard aluminum solution was prepared by dissolving 0.1759 g of aluminum potassium sulphate ($AlK(SO_4)_2.12H_2O$) in water, and diluting to 1 liter. This solution contained 10 μg of aluminum per milliliter.

The buffer solution was a saturated solution of ammonium carbonate, adjusted to pH 9.5–10 with ammonium hydroxide.

The reagent was a 1% w/v solution of oxine in carbon tetrachloride. Other reagents included a 1% w/v solution of 8-hydroxyquinaldine in chloroform, a 10% w/v aqueous solution of potassium cyanide, and diethylammonium diethyldithiocarbamate.

All other chemicals used were of analytical reagent grade.

Development of the Method

Preliminary Studies

Oxine forms extractable complexes with over 40 elements. Of the trace elements usually to be found in uranium metal and its compounds, almost all will form extractable

[1]*Manuscript received January 30, 1961.*
Contribution from the Research and Development Division, Eldorado Mining and Refining Limited, Ottawa, Ontario.

oxinates. It was therefore essential to remove or complex all elements whose oxinates are extracted at the pH at which the aluminum is extracted. Of the reagents studied for either extraction or complexing of these elements, 8-hydroxyquinaldine appeared to be the most suitable. Merritt and Walker (3) have found that 8-hydroxyquinaldine does not complex aluminum, due to steric hindrance, but otherwise behaves almost like oxine in its reactions. Hynek and Wrangell (4) used this reagent for the extraction of interfering elements from a solution buffered at pH 9.2. It was found in this laboratory that this pH was not critical. The removal of interfering elements with this reagent, at a pH of 9.5–10, was studied.

Aluminum Calibration

For extraction purposes, oxine is generally used in chloroform solution. In the presence of uranium a carbon tetrachloride solution was found to give lower blank values, and this solvent was used throughout these experiments.

A calibration curve was determined by adding known amounts of aluminum to an ammonium chloride – ammonia buffer solution, and extracting with 20 ml of the oxine solution. Absorbance measurements of the extracts were determined at 430 mμ, using 5-cm cells. A straight-line calibration curve was given.

Complexing of Uranium

Since uranium precipitates in an ammonia – ammonium chloride solution, various reagents were tried in order to keep the uranium in solution at pH 9–10. Ammonium tartrate and citrate, while keeping the uranium in solution, inhibited extraction of aluminum. Uranium is soluble in ammonium carbonate solutions at this pH, and this solution was found to have no effect on the extraction of aluminum. A saturated solution of ammonium carbonate, adjusted to pH 9.5–10 with ammonium hydroxide, was used, both to keep the uranium in solution and as a buffer solution.

Interfering Elements

Elements found in uranium metal and its compounds which are likely to interfere in the determination are cobalt, manganese, nickel, zinc, copper, and iron. A study of the removal of these elements by extraction with a 1% solution of 8-hydroxyquinaldine in chloroform was carried out.

Known amounts of these elements were added to ammonium carbonate – ammonia buffer solutions, containing uranium and 25 μg of aluminum. Extraction with 8-hydroxyquinaldine showed that up to 100 μg of cobalt, nickel, zinc, copper, lead, and iron could be extracted without effect on the aluminum recovery (Table I). Manganese, however, was

TABLE I

Extraction of elements with 8-hydroxyquinaldine

Element added	Amount added, μg	Aluminum* recovered, μg
Cobalt	100	24
Nickel	100	25
Zinc	100	25
Copper	100	25
Iron	100	25
Lead	100	26
Manganese	10	27
"	20	30
"	100	63

*Twenty-five micrograms of aluminum added in each case.

not extracted by the 8-hydroxyquinaldine, but was extracted by the oxine, giving large positive errors in the aluminum determination. The valency state of the manganese had no effect on its extraction. Small amounts of manganese (5 μg) had little or no effect on the aluminum determination. Larger amounts had to be removed.

Removal of Manganese

There appeared to be no reagent available which would complex manganese under the conditions of the experiments. A study of reagents suitable for its extraction prior to the determination of aluminum showed diethylammonium diethyldithiocarbamate to be the most suitable. This reagent had no effect on the recovery of aluminum. Sample solutions containing more than 5 μg of manganese were extracted with chloroform after the addition of solid diethylammonium diethyldithiocarbamate. Extraction of other interfering elements was completed by extraction with 8-hydroxyquinaldine.

In order to ensure the complete removal of deleterious effects of interfering elements which may not have been completely removed by the extraction with 8-hydroxyquinaldine, potassium cyanide solution was added before addition of the oxine solution. Potassium cyanide did not affect the extraction of aluminum.

Preparation of Samples

Samples of uranium metal and uranium compounds were dissolved in a 3:1 mixture of hydrochloric and nitric acids. The solutions were then boiled to remove oxides of nitrogen, and diluted to a suitable volume.

Recommended Procedure

Dissolve and dilute the sample as described under Preparation of Samples. Transfer by pipette an aliquot containing 5–50 μg of aluminum into a 125-ml, separatory funnel. Add 30 ml of the buffer solution and mix. If the aliquot contains more than 5 μg of manganese, add 1 g of diethylammonium diethyldithiocarbamate, mix thoroughly, and extract with 10-ml portions of chloroform until the extracts are colorless. Add 5 ml of the 8-hydroxyquinaldine solution and shake for 2 minutes. Allow the phases to separate and discard the organic phase. Repeat this extraction until little or no color is given in the organic phase. Extract the aqueous phase with 3×10-ml portions of chloroform. Discard the extracts. Add 5 ml of potassium cyanide solution and mix. By pipette, add 20 ml of the oxine solution into the funnel and shake the mixture for 2 minutes. Allow the phases to separate and filter the organic phase through a coarse, dry filter paper. Measure the absorbance of the extract at a wavelength of 430 mμ, using 5-cm cells, with the spectrophotometer set to zero on a blank.

Calibration Procedure

Measure from a burette 0-, 1-, 2-, 3-, 4-, and 5-ml volumes of the standard aluminum solution into 125-ml separatory funnels, and carry them through the procedure. Plot absorbance readings against micrograms of aluminum. Beer's law is obeyed.

Results and Precision

Table II presents some typical results obtained on uranium metal samples, together with results obtained on a New Brunswick Laboratories Analysed Sample (No. 16) of uranium metal. Also shown is the precision obtained on samples of uranium metal containing low and high quantities of aluminum.

The method gave a standard deviation of 0.8 p.p.m. aluminum at the 13 p.p.m. level, and the 95% confidence limits on a single determination of ±1.7 p.p.m. At the 600 p.p.m. level these were 7.5 and ±16 p.p.m. respectively.

TABLE II

Results obtained on samples of uranium metal and uranium dioxide

Sample		Aluminum, p.p.m.
NBL No. 16*		12, 12, 14, 13, 14, 13
UO_2	A	4, 4
	B	3, 3
	C	60, 61
	D	52, 54
Uranium	A	573, 570, 568, 572, 575
Metal	B	935, 945
	C	14, 14, 13

*NBL certificate, 10 p.p.m. (spectrographic analysis).

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A STUDY OF THE EMISSION INTENSITIES OF Cr LINES FROM SHOCKED GASES CONTAINING $Cr(CO)_6$[1]

S. H. Bauer, John H. Kiefer, and Brian E. Loader

ABSTRACT

Argon–nitrogen mixtures (9/1) were inoculated with traces of $Cr(CO)_6$ (0.026%) and shocked to temperatures in the range 3500° K to 5700° K. The intensities of a violet line ($^7P_2 \rightarrow {}^7S_3$) and a triplet of green lines ($^5P_{3,2,1} \rightarrow {}^5S_2$) were measured concurrently, with a dual-channel spectrograph. Exclusive of runs for which $T > 5000°$ K, the variation of intensity with temperature was brought to agreement with theoretical predictions under the assumption that the N_2 vibrations, the dissociation of $Cr(CO)_6$, and the ionization of Cr attained their equilibrium states, but allowance had to be made for self-absorption. The dependence of the rise times on temperature suggest that the excitation and ionization of Cr is predominantly due to collisions with electrons. The over-all shapes of the recorded light pulses were qualitatively accounted for. It is suggested that extraneous background radiation contributed to the measured intensities for samples shocked to temperatures above 5000° K.

INTRODUCTION

Studies of the excitation of atomic energy states by collisions with heavy particles in the thermal energy range have been limited experimentally by the difficulty of preparing sufficiently intense beams of incident atoms or molecules and theoretically by the inherent complexity of the potential energy hypersurfaces which must be considered in the treatment. Efficiencies for energy transfer in de-excitation processes have been measured for a number of target atoms from the quenching of radiation due to the introduction of a variety of incident molecules. The cross sections (Q) for the forward and reverse processes are, of course, related. For particles which have a high relative kinetic energy (primed),

$$Cr' + Ar' \leftrightarrows Cr^* + Ar,$$

let the p' be the momentum of relative motion prior to excitation and p of the excited pair. Then (1, p. 417),

$$[1] \qquad g_{Cr}g_{Ar}p'^2Q' = g_{Cr^*}g_{Ar}p^2Q,$$

where the g's designate the statistical weights, as indicated. Since $p'^2 > p^2$, the cross section for the superelastic process is generally greater than for the excitation process. However, the cross sections for collision-induced electronic transitions are very small unless the change of internal energy is very small. Per impact, the transition probability is low if

$$[2] \qquad a|\Delta E|/hv \gg 1,$$

where a is the range of interaction between the atoms, and v is their relative velocity, since such an event takes place at near-adiabatic conditions. Massey and Burhop (1, p. 515) suggest that the cross section for excitation should then vary as $\exp(-Ca|\Delta E|/v)$ and consequently would increase exponentially with temperature, for a thermalized system.

Investigations of the luminous front in shocked gases provide data on excitation processes in thermalized systems where the average relative kinetic energies of the participating particles are less than one volt. A theory for the excitation of atoms by

[1]*Manuscript received October 24, 1960.*
Contribution from the Department of Chemistry, Cornell University, Ithaca, New York. Presented at the Symposium on Fundamental Aspects of Atomic Reactions, McGill University, Montreal, Que., September 7, 1960.

collisions at elevated temperatures has been outlined by Nikitin (2). In this paper, we describe preliminary work on the excitation of resonance lines in chromium, introduced in trace amounts as $Cr(CO)_6$ in argon–nitrogen mixtures and shocked to temperatures from 3000–5500° K. The possible utility of chromium emission intensity ratios for the estimation of shock temperatures has been analyzed previously (3). Wilkerson (4) obtained time-resolved Cr(I) and Cr(II) emission spectra from incident and reflected shocks in Ne which had been inoculated with 0.3% of chromium carbonyl. Recent studies (5) of the delayed luminosity in highly purified xenon as induced by a shock have proved both interesting and puzzling, since the current predictions suggest that the potential curves for two xenon atoms do not cross over the energy range of collisions which are prevalent in shocked gases at 6000° K. It now appears that the diameter of the shock tube, the composition of the tube walls, and the purity of the driver gas play important roles in determining both the electrical and optical structure of the shock front.

The sensitivity to slight impurities, which appears in accentuated form when one measures the electrical conductivity of shocked gases, is due to free electrons, the concentration of which is probably determined by the lowest ionization potential of any impurity present in the gaseous sample and by the nature of the shock-tube walls. It also seems likely that these electrons play a dominant role in collision-induced electronic excitations. For the excitation of electronic states, the collision cross section becomes appreciable only when the *speed* of the incident particle approaches that of the atomic electron involved in the transition (1, p. 515). Hence, at the temperatures developed in the shocked gases, the average energy per pair is too low to provide an appreciable transition probability for excitation by atom–atom collisions. However, in that energy range, the efficiency of excitation by collision with electrons is much greater, perhaps by a factor of 10^3.

Insofar as there is *structure* to the shock front, no single measure of temperature can be specified. The very difficult problem of determining the velocity distribution of the electrons in this region has yet to be solved. One may argue, however, that beyond a distance of the order of 10 mean free paths following the steep pressure gradient in the shock front the electrons will have attained translation equilibrium and would then possess the same translational temperature as the massive particles present.

SPECTROSCOPIC MEASUREMENT OF SHOCK TEMPERATURES

The gas temperature behind a shock wave may be computed from the measured shock speed, the composition of the gas being subjected to the shock, and its initial temperature and pressure, provided one assumes that thermodynamic equilibrium is attained in the system for specified end products. One must postulate not only definite chemical species but also their allowed states of excitation, so that the thermodynamic state of the shocked gas is determined by a temperature, a density, and a set of mole fractions. When the chemical kinetics of a system are being studied in a shock tube, such detailed information and its dependence on distance behind the shock front are not available. However, it is meaningful to consider the translational temperature of the gas and to inquire whether the temperatures which may be associated with rotations, vibrations, and electronic states are in equilibrium with translation.

Various techniques have been developed for directly estimating temperatures in very hot gases. Stark shifts (6) and the broadening of spectral lines (7) have been used for very strong shocks and gases heated by electrical discharges. Gaydon and co-workers

(8) described a modified form of the sodium-line-reversal technique to deduce temperatures with an estimated accuracy of ±20° C. Although this method is capable of higher precision when long measuring times are available as in steady-state conditions for flames (9), it is accompanied by considerable difficulties when the data must be recorded with microsecond resolution. To produce a sufficiently dense suspension of sodium chloride particles in the gas to be shocked, they found it necessary to pass the sample through a hot tube (~800° C) over the molten salt. Very few gases of chemical interest may be treated in this manner. It is interesting to note that electronic temperatures deduced for flames by the reversal technique utilizing various spectral lines from iron, lead, etc. (10), were found to be higher than the maximum theoretical temperature for the specified composition. The origin of these excessive temperatures is not known, but it is surmised that they arise partly from chemical excitations.

The relative emission intensities of two atomic lines characteristic of a trace impurity are a measure of temperature, provided one may assume that the electronic states of the emitter have attained equilibrium with translation or vibration. Then, the intensities can be readily converted to a temperature from the known location of the corresponding upper states and the absolute f numbers or by a calibration procedure. If the concentration of emitter is sufficiently low so that self-absorption may be ignored, the results are independent of emitter density (3). Sobolev *et al.* (11), tested this procedure with lithium chloride and sodium chloride as trace emitters but obtained badly scattered results due to self-absorption, inhomogeneous gas distribution, etc.

To avoid the difficulty of working with crystallites of a nonvolatile material, we measured the emission intensities of the resonance lines of chromium at λ4290 and λ5206, derived from known concentrations of traces of chromium carbonyl in Ar–N_2 mixtures. Manganese, iron, tungsten, and nickel carbonyls can be similarly used as sources of metal atoms. Flame spectra (2200–3200° K) indicate that the spectral sensitivities of these chromium lines are of the order of 10^{-2} of that of NaD. We thus have controlled concentrations, with pressures which may be as high as 0.3 mm Hg of emitter gas.

THEORY

A rough numerical estimate shows that in the shock front the change of intensity with time is indeed a measure of the rates of excitation of the chromium atoms, that is, of the process:

$$Cr_{(g)}[a, {}^7S] + X_{(g)} \xrightarrow[k_z]{} Cr_{(g)}^*[z, {}^7P \text{ or } z, {}^5P] + X$$

and not that due to the rate of dissociation of the carbonyl. The enthalpy change at 0° K for the reaction

$$Cr(CO)_{6(g)} = Cr_{(g)} + 6CO_{(g)}, \qquad \Delta H^\circ_0 = 173.23 \text{ kcal/mole} \tag{12a}.$$*

It follows that the average ⟨Cr—CO⟩ bond dissociation energy is 28.9 kcal. An order of magnitude for the unimolecular rate constant for the dissociation

$$Cr(CO)_n^* \xrightarrow[k_{uni}^{(n)}]{} Cr(CO)_{n-1} + CO$$

is:

$$k_{uni}^{(n)} \simeq 10^{18} e^{-29,000/RT}, \text{ sec}^{-1}. \tag{3}$$

**The authors reported that the standard heat of formation at 298.2° K of $Cr(CO)_{6(g)}$ is −257.57±0.51 kcal/mole. Correction for the heat of sublimation of $Cr(CO)_6$ (ΔH°_{sub} $Cr(CO)_6$ = 17.18 kcal, as reported in reference 12b), the heat of sublimation of $Cr_{(s)}$ (ΔH°_{sub} $Cr_{(s)}$ = 95 kcal/mole, as reported in reference 12c), and the heat of formation of 6CO molecules leads to ΔH°_{298} = 176.90 kcal for the indicated dissociation reaction.*

If one postulates that on the average this value is typical of each of the six steps, the time required for $(Cr)/(Cr)_{final}$ to reach 0.9 at 1000° K is $t_{0.9} \approx 10/k_{uni}^{(n)} \approx 10^{-10.7}$ sec. This is much less than the response times of currently available recording apparatus. For all practical purposes, the metal carbonyl is completely dissociated in the forward portion of relatively weak shocks.

The spectral radiancy from a distributed emitter in thermal equilibrium, of optical depth X ($= pL$, atm cm) and spectral absorption coefficient P_ω(atm^{-1} cm^{-1}), is (13)

$$[4] \qquad R_\omega = R^\circ_\omega[1-\exp(-P_\omega X)].$$

The black-body radiancy at the specified temperature is given by

$$[5] \qquad R^\circ_\omega = 2\pi hc^2\omega^3[\exp(hc\omega/kT)-1]^{-1}.$$

Since the spectrometer–phototube–oscilloscope system integrates radiation over a selected line, with central position at ω_1, one should use the integral of the spectral absorption coefficient. For a Gaussian shape,

$$[6] \qquad P_\omega X = \alpha \exp\left[-\left(\frac{\omega-\omega_1}{\beta}\right)^2\right]; \qquad \int_{line} P_\omega d\omega = \frac{\sqrt{\pi}\alpha\beta}{X}.$$

However,

$$[7] \qquad \int_{line} P_\omega d\omega = \frac{\pi e^2}{mc^2}\frac{N_l}{p} f_{l\to u}\,(1-e^{-hc\omega_1/kT}),\ \text{cm}^{-2}\ \text{atm}^{-1}$$

where

$f_{l\to u}$ = oscillator strength for the transition from the lower (l) state to the upper (u) state (for Cr, refer to the report by Hill and King (14)),

$$\omega_1 = \frac{E_u - E_l}{hc},$$

N_l = number of molecules of emitting species per cubic centimeter, in the lower state,

p = pressure (atm) of emitting species.

Whence,

$$[8] \qquad \alpha = \frac{L}{\sqrt{\pi}\beta}\frac{\pi e^2}{mc^2} N_l f_{l\to u}\,(1-e^{-hc\omega_1/kT}).$$

If $P_\omega X$ is sufficiently small, the exponential in [4] may be expanded and the terms integrated (for the Gaussian shape), provided one assumes that $R^\circ_{\omega_1}$ is constant over the narrow band of wave lengths which is transmitted by the exit slit.

$$[9] \qquad V_{\omega_1} = K_{\omega_1} R^\circ_{\omega_1}\sqrt{\pi}\alpha\beta[1-\alpha/2\sqrt{2}+\ldots]$$

$$= K_{\omega_1}\frac{2\pi^2 he^2\omega_1^3}{m} N_l L f_{l\to u} e^{-hc\omega_1/kT}[1-\alpha/2\sqrt{2}+\ldots]$$

K_{ω_1} is a transmission-amplification constant which relates the oscilloscope-voltage output to the intensity of ω_1 picked up by the spectrometer. The term in α in the bracket is a measure of self-absorption; it is density and temperature dependent.

[10] $$N_l = \rho \frac{g_l}{Q} e^{-E_l/kT}$$

where Q is the total partition function, with the ground state taken as the zero; ρ is the total emitter density, in molecules per cubic centimeter.

[11] $$V_{\omega_1} \propto \frac{\rho g_l}{Q} f_{l\to u} \exp\left(-\frac{hc\omega_1 + E_l}{kT}\right)$$

For a given spectrometer setting, a plot of $\ln [V_{\omega_1} Q/\rho]$ vs. $1/T$ should be a line with slope $-(hc\omega_1 + E_l)/k$. If appreciable ionization takes place, the concentration of atoms in state l will be depleted by an amount which depends sensitively on the temperature, as given by the Saha equation.

Provided self-absorption is in fact negligible, the 'two-color temperature' (15) is given by the ratio

[12] $$\frac{V_{\omega_1}}{V_{\omega_2}} = \frac{K_{\omega_1}}{K_{\omega_2}} \frac{g_{l_1} f_1}{g_{l_2} f_2} \exp\left[-\frac{E_{u_1} - E_{u_2}}{kT}\right]$$

since $E_{l_1} + \omega_1 = E_{u_1}$, etc. The unknown ratios may be lumped into a single constant which may be determined by calibration with shocks of measured speed under idealized conditions.

The observed rise time for each line depends on many factors. In addition to the response time of the recording electronics and particle passage time past the cone of observation, the rate of approach to the 'equilibrium' emission intensity may be limited by the vibrational and dissociation relaxation of nitrogen, if present, and by the finite rates for excitation and ionization of the chromium atoms. Since $dV(t)/dt \propto |f_{u\to l}| dN_u/dt$ and $V_e \propto g_l f_{l\to u} (N_u)_e / g_u$,

[13] $$\left[\frac{1}{V_e}\frac{dV(t)}{dt}\right]_{corr} \propto \frac{1}{(N_u)_e}\frac{dN_u}{dt}$$

is an experimental 'rise time' characteristic of the atomic state and of the excitation processes. There are at least three distinct processes which populate the specified upper state:

(a) excitation from a lower state by collision with a heavy particle:

$$\mathrm{Cr}(i) + \mathrm{Ar}' \xrightarrow[k_i]{} \mathrm{Cr(u)} + \mathrm{Ar}$$

(b) excitation by collision with an electron:

$$\mathrm{Cr}(j) + \mathrm{e}^- \xrightarrow[k_j]{} \mathrm{Cr(u)} + \mathrm{e}^-$$

(c) radiative recombination with an electron:

$$\mathrm{Cr}^+ + \mathrm{e}^- \xrightarrow[k_e]{} \mathrm{Cr(u)} + h\nu.$$

The total rate is the sum over all states i and j.

The mechanism for ionization in shock fronts has been discussed by a number of authors (16, 17). Electron–atom processes have the largest cross sections. This is also the case for excitation reactions (b) (1, p. 515; 18). Some electrons are initially present in the unshocked gas; some of these may have been generated photoelectrically by

photons downstream from the shock front, others by residual radioactivity. Their concentration is rapidly increased when the gas is compressed by the shock. Because of their low mass, these electrons diffuse ahead of the shock front and establish a double layer across which there exists a considerable potential drop. In the co-ordinates of the moving shock, rapidly moving atoms enter from the upstream side and collide with relatively energetic electrons to produce excited atoms and more electrons.

The depopulation of the upper state can occur via:

(a') the reverse of (a):

$$\mathrm{Ar} + \mathrm{Cr(u)} \xrightarrow[k_i']{} \mathrm{Cr}(i) + \mathrm{Ar}'$$

(b') the reverse of (b):

$$\mathrm{e}^- + \mathrm{Cr(u)} \xrightarrow[k_j']{} \mathrm{Cr}(j) + \mathrm{e}^-$$

(d) radiation:

$$\mathrm{Cr(u)} \xrightarrow[f_{u\to i}]{} \mathrm{Cr}(i) + h c\omega_i$$

(e) ionization from the excited state:

$$\mathrm{Cr(u)} + \mathrm{e}^- \xrightarrow[k_x]{} \mathrm{Cr}^+ + 2\mathrm{e}^-.$$

Electrons are also produced via:

(f) ionization from all other states:

$$\mathrm{Cr}(j) + \mathrm{e}^- \xrightarrow[k_+]{} \mathrm{Cr}^+ + 2\mathrm{e}^-.$$

Let n be the concentration of electrons in the shocked gas at $t = 0(+)$ and $c(t)$ the additional electrons generated via (e) and (f). For the early stages ($t < t_{\frac{1}{2}}$), one may write

$$\frac{d(n+c)}{dt} \simeq [k_x(\mathrm{Cr_u}) + k_+(\mathrm{Cr}_j) - k_c(\mathrm{Cr}^+)](n+c).$$

Then, since $(\mathrm{Cr}^+) \ll (\mathrm{Cr})$, an average value of the ionization rate constant may be used,

$$(n+c) \simeq n \exp\,[\langle k\rangle \rho_{\mathrm{Cr}} t]; \qquad [14]$$

i.e., the electron concentration initially rises exponentially.

It is very likely that under shock conditions (b') and (e) are more effective in depleting the upper state than are (a') and (d). We estimated that in the experiments described below the mean time between collisions (kinetic-theory cross sections) is between 1×10^{-9} sec to 4×10^{-10} sec, whereas the radiative lifetimes of the ^7P and ^5P states of chromium are of the order of 10^{-8} sec. For the net rate of population of the upper state, assuming that atom–electron processes dominate,

$$\frac{d(\mathrm{Cr_u})}{dt} \simeq (n+c)[k_j(\mathrm{Cr}_j) + k_c(\mathrm{Cr}^+) - (k_x + k_j')(\mathrm{Cr_u})]. \qquad [15]$$

Since, at $t = 0(+)$, $k_j(\mathrm{Cr}_j)^\circ \gg (k_x + k_j')\ (\mathrm{Cr_u})^\circ$, the population of the excited state may 'overshoot' its equilibrium value and thus produce a maximum in the emitted

intensity vs. time curve. For comparable rate constants, say $(k_x+k_j') = k_j(1+\alpha)$ with α small, $t_m = 1/k_j$. Whether the maximum is experimentally observable will depend on the temporal behavior of $(n+c)$. If the latter reaches its equilibrium value rapidly, the maximum will be recorded and will become more pronounced in amplitude as $(k_x+k_j')/k_j$ decreases.

There are insufficient data at present to permit a complete kinetic analysis of the above complex mechanism. However, as a preliminary approach, assume that the net rate of excitation to the upper level at the shock front may be expressed in terms of a single rate constant. Let

$$\left(\frac{dN_u}{dt}\right)_{net} = \rho_{Cr}{}^{n} A \exp\left(-E_u^*/kT\right). \tag{16}$$

Hence,

$$\frac{1}{\tau_0} \equiv \frac{1}{V_e}\left(\frac{dV}{dt}\right)_{initial} = \frac{AQ}{g_u} e^{-(E_u^*-E_u)/kT} \rho_{Cr}{}^{n-1}. \tag{17}$$

E_u^* has the significance of an activation energy* and corresponds to the energy at the crossover point of the unperturbed potential function curves for Cr-incident particle and $Cr_{(u)}$-incident particle. A plot of $\ln [1/QV_e\rho^{n-1}(dV/dt)_i]$ vs. $1/T$ should give a line with a slope $-(E_u^*-E_u)/k$. If process (a) dominates, the slope should be steep, whereas under conditions where (b) and (c) control the rate, $E_u^* \approx E_u$.

In the above formulation, it has been assumed that the temperature of the shocked gas was unambiguously defined and independent of time. This is the case for samples which consist solely of argon with traces of chromium carbonyl, since the depletion of energy by the dissociation of $Cr(CO)_6$ and ionization of Cr results in a temperature drop of no more than 10° K at the highest temperatures studied. However, when the ambient gas contains an appreciable amount of N_2 (circa 10%), the shock front consists of several relaxation zones, which are superposed, due to vibrational excitation and dissociation of the nitrogen, as well as the excitation and ionization of the chromium. In the samples shocked to temperatures above 4700° K, an appreciable fraction of the nitrogen is dissociated at equilibrium. Preliminary measurements by Byron (private communication) gave for the dissociation rate constant at 7000° K

$$N_2 + Ar \underset{k^d}{\overset{k^a}{\rightleftharpoons}} 2N + Ar$$

$$(dN/dt)_{initial} = k^d(N_2)(Ar); \qquad \begin{aligned} k_{Ar}{}^{d} &= 2.8\times10^{8}, \text{ (mole/cc)}^{-1}\,\text{sec}^{-1} \\ k_{Ar}{}^{a} &= 1.7\times10^{14}, \text{ (mole/cc)}^{-2}\,\text{sec}^{-1} \end{aligned}$$

(As a third body, N_2 appears to be about 2.5 times as effective as Ar. In our system, however, the Ar rate dominates.) We estimated that at 5000° K, in a 10% N_2 – 90% Ar mixture, the half time for the attainment of dissociation equilibrium is 80 milliseconds.

*The product of the time elapsed between ionization onset and the attainment of its maximum value and the initial pressure of the shocked gas ($\tau_i p_1$) was found to decrease approximately linearly with increasing Mach number, for air (Y. Manheimer-Timnat and W. Low. J. Fluid Mech. 6, 449 (1959); B. Niblett and V. H. Blackman. J. Fluid Mech. 4, 191 (1958)). Generally, the Mach numbers correlate with the initial pressures. It is suggested, therefore, that these ionization–relaxation times might be more uniquely interpreted in terms of eq. [17].

EXPERIMENTAL

The following experiments were performed in a stainless-steel tube, 6-in. I.D., Fig. 1.

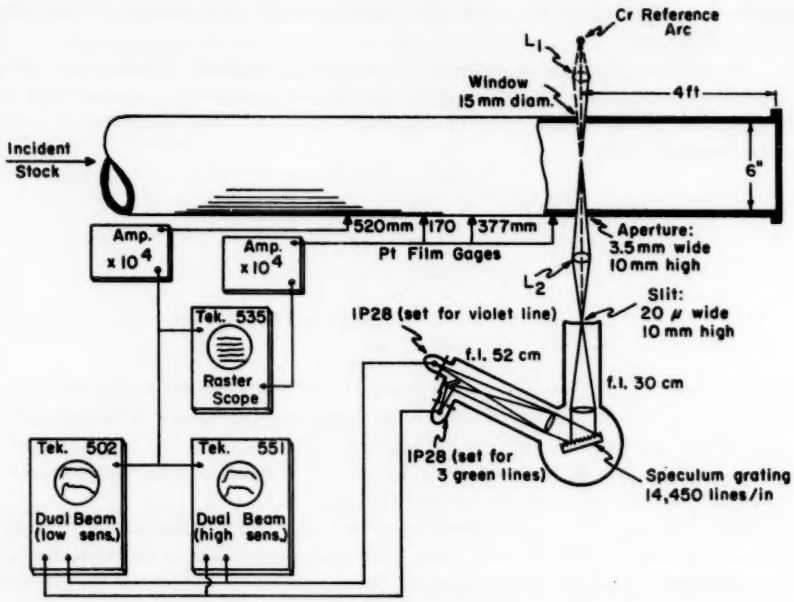

FIG. 1. Schematic arrangement of the optics and a block diagram of the electronic components. The spectrograph had an entrance slit 20 μ wide and 10 mm high. The dispersion at a camera focal length of 52 cm was 30 Å/mm. The exit slit for the violet line was set at 70 μ and for the green lines at 170 μ.

The driver section was 8 ft long. The windows through which the luminosity was observed were located 18 ft downstream from the diaphragm. Beyond the windows, the tube extended 4 ft, so that ample time was provided to avoid confusion with the reflected shock. The diaphragms were of half-hard brass, 0.032 in. thick, precision scribed through half their thickness. In all of the experiments described, the diaphragms were broken by exceeding the predetermined breaking pressure. Depending on the batch of sheet stock used, this was reproducible to within 5 lb. The windows were of quartz, and the aperture for observing the emitted light was limited to a section 3.5 mm wide and 10 mm high. Gauges for detecting shock arrival times were thin platinum films supported on Pyrex, placed at the indicated locations. Hydrogen was used as the driver gas at pressures of 200 to 230 p.s.i. The temperature of the shock was controlled by the pressure of the driven gas, which consisted of argon plus chromium carbonyl or a mixture of argon and nitrogen (9/1) plus the chromium carbonyl. The shock tube was evacuated with a 4-in. diffusion pump to 10^{-5} mm Hg; the leak rate was about one-half micron per minute.

We found it essential to premix the chromium carbonyl with the argon or argon–nitrogen mixture to insure uniformity of the sample. The mixtures consisted of chromium carbonyl vapor as derived from the solid maintained at room temperature and expanded into a 50-gal glass-lined tank to a pressure of 70 μ. To this, 27 cm of the carrier gas

was added and the mixture allowed to stand for several days prior to use. The concentration of chromium carbonyl in the test samples was 0.026%. Total pressures of the driven gas ranged from 0.65 cm to 3.15 cm to cover the full range of shock temperatures. Shock speeds were measured from a raster display. The optical arrangement is also diagramed in Fig. 1. The two channels of the grating spectrograph (dispersion, 30 Å/mm) were aligned with a chromium arc so set on the opposite side of the tube as to focus to a virtual source in the center of the tube. The violet line at λ4289.7 ($a\ ^7S_3 \leftarrow z\ ^7P_2^0$) was sent along the central axis of the receiver section and the receiving slit set to admit a region in wave length roughly twice the width of the line. The small side mirror was then positioned so that the green triplet [λ5208.4, 5206.0, 5204.5 ($a\ ^5S_2 \leftarrow z\ ^5P^0_{3,2,1}$)] passed through the side receiver; this slit also was set to collect a little more than the triplet (19).*

The two photomultipliers were powered by a single well-regulated voltage supply, and the output of these tubes was split and sent to two dual-beam oscilloscopes, so that the intensity of the two lines could be recorded simultaneously at two sensitivity levels. This was necessary because the level of emission intensity was not always predictable. The time constant for the phototubes, amplifiers, and recording oscilloscopes was of the order of 4 μseconds. In some of the experiments to be described below, another set of phototubes was used. These were constructed with cathode followers which gave an electronic time constant of 0.8 μsecond but were of limited utility since they were easily saturated. The experimental 'rise time' was not limited by the 2- to 3-μsecond passage time of the gas past the cone of observation as viewed by the spectroscope. Typical shock parameters are summarized in Table I for a high-temperature shock and for one

TABLE I

Shock parameters for typical experiments

(Driver: H_2, 200–220 p.s.i., gauge. Mixture: Ar (90%), N_2 (10%), $Cr(CO)_6$ (0.026%))

	Run No. 51		Run No. 50	
Initial sample pressure (total), cm at T_1 = 300.5° K	3.15		0.65	
Initial density (total), in mole cc^{-1}	1.68×10^{-6}		3.38×10^{-7}	
Incident shock speed, in mm/μsec	1.974		2.556	
Incident shock T_2 (no N_2 vib. or dissoc.)	3560° K		5789° K	
p_2, cm	139		48.3	
ρ_2 (total), mole cc^{-1}	6.74×10^{-6}		1.35×10^{-4}	
Density of Cr atoms $(\rho_2)_{Cr}$, mole cc^{-1}	1.68×10^{-9}		3.48×10^{-10}	
Particle speed, in mm/μsec	1.21		1.79	
Particle passage time past cone of observation, in μsec	2.89		1.95	
Theoretical interval between shock front and contact interface, in μsec	920		700	
Light intensity at t_m	Green	Violet	Green	Violet
(1000 v on dynodes, voltage output across 10K load resistor)	0.888	1.475	3.99	7.54
$[(1/V_m)(dV/dt)_i]^{-1}$ as measured, in μsec	34.5	30	13.0	13.9
Approx. width of light pulse (lab time), in μsec	480		360	

near the lower end of the temperature range studied. Figure 2 shows traces of typical oscillograms.

**The author gives energy levels and frequencies for chromium. Note that the $a\ ^5S_2$ state is 7593 cm^{-1} above the ground $a\ ^7S_3$ state.*

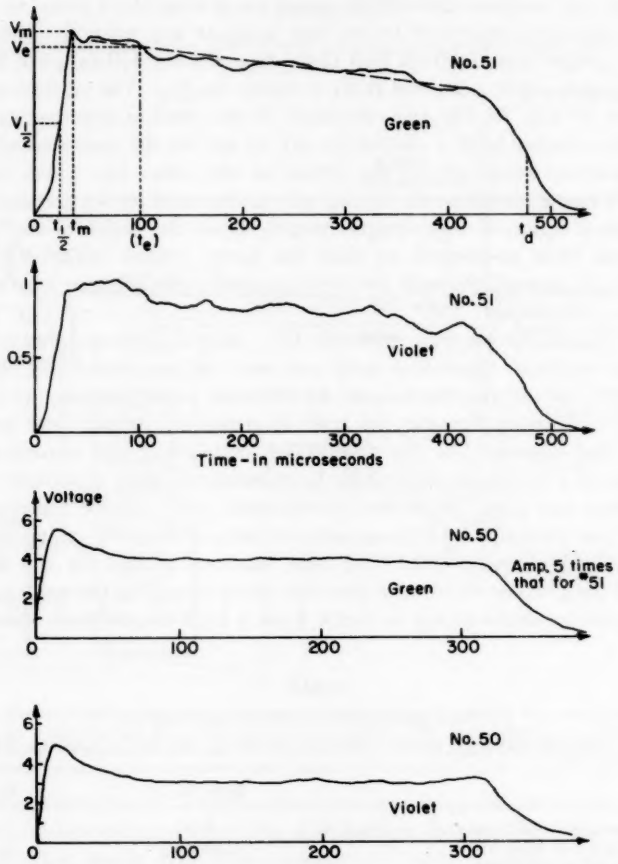

FIG. 2. Tracings of the oscilloscope records of the violet and green line photomultiplier outputs for two runs. Refer to Table I for a summary of the experimental conditions. The characteristic features of these voltage variations are designated in the top curve.

SUMMARY OF OBSERVATIONS

One of the first experiments performed was a spectroscopic examination of the total light emitted. The double-channel photomultiplier unit was replaced by a camera. In order to enhance the intensity of the emitted light, a fairly high concentration of chromium carbonyl was introduced and the spectrum taken in the reflected shock region. The plate showed a very large number of lines. On careful examination, all of the lines were identified and proved to be due to Cr(I). No other lines nor background intensities were observed under these conditions. On these plates, the lines of interest appeared reversed, which suggested that the amount of carbonyl should be reduced from the initial 300 μ to roughly 3 μ.

In another crucial experiment, the initiation of light emission was checked against the arrival time of the shock at the window. For this measurement, the phototube circuitry had a rise time of 0.8 μsecond. To within 1 microsecond, the incidence of light

emission coincided with the arrival time of the shock front at the central line of the viewing port; further inspection of the traces suggested that the luminosity started perhaps half to 1 microsecond earlier.

The oscillogram records show a number of features for analysis. Specifically, the following will be considered:

(*a*) for each line, the variation of intensity with shock temperature and density,
(*b*) the rise time of light emission at the shock front and its dependence on temperature, on the composition of the ambient gas (presence of 10% N_2 diluent), and on the specific levels involved, and
(*c*) the general shape of the emission output as a function of time; in particular, the presence of an initial maximum and the duration of the pulse.

ANALYSIS OF THE DATA

Since most of the shocks were run with the 9/1 Ar/N_2 mixture, the question arose as to whether the effective temperature in the shock front should be computed with the vibrational contribution of the nitrogen included or deleted. From Blackman's data (20), we estimated that the vibrational relaxation time for run No. 50 was 7 μseconds, and for No. 51 it was 10 μseconds (assuming no difference in efficiency between Ar–N_2 and N_2–N_2 collisions), with intermediate periods for the other runs. These times are more than twice that required for the particles to pass the cone of observation but about half of the observed rise time (lab. co-ordinates) for emission. Were the populations of the $^7P_2{}^0$ and $^5P^0{}_{3,2,1}$ states to rapidly attain equilibrium with the vibrational temperature (cf. Clouston and Gaydon (8)), the rise time for emission would be considerably shorter than that observed. The lag in the excitation of vibrations should at most affect the *initial* slope of the emission-time curve but should have no influence on the slope at the half-height position ($V_{\frac{1}{2}}$ at $t_{\frac{1}{2}}$, Fig. 2). In view of our estimate of the half time for the attainment of dissociation equilibrium, the temperatures used in Figs. 3–5 were computed on the assumption of partial equilibrium; i.e., we included the excitation of vibrations in N_2 (these reduced the translational temperatures by 60–180° K, over the range 3500–5800° K), the complete dissociation of the chromium carbonyl ($\sim$ 8° K), and the ionization of chromium atoms (0–7° K). The ionization of argon is negligible even at the highest temperatures.

An analysis of a number of runs is presented to illustrate features *a*, *b*, and *c*. In Figs. 3 and 4, eq. [11] was applied to the recorded intensities of the violet and green lines at t_m and t_e, respectively. The ordinates were computed on the assumption that the population of the upper states was proportional to the total chromium concentration. Within the scatter of the data, the slopes of the two sets of curves are independent of the time, for $t > t_m$, and for the lower third of the range in $1/T$ the observed slopes check the theoretical values. At the upper temperatures, the recorded intensities fall below the theoretical curves by a factor which increases with the temperature. This departure is considerably greater for the green line than for the violet lines and is more evident at t_e than at t_m. The same data were replotted on the assumption that the populations of the upper states were proportional to the concentration of chromium atoms which remain unionized; i.e., the abscissa was $\log [V_m Q/7\rho_{Cr}(1-x)]$, where x is the percentage ionized at the equilibrium shock temperature and density, as computed by means of the Saha equation; considerable ionization is present at equilibrium for $1/T \leqslant 2.1\times10^{-4}$. The points were found to lie on the theoretical slopes over the lower two thirds of the

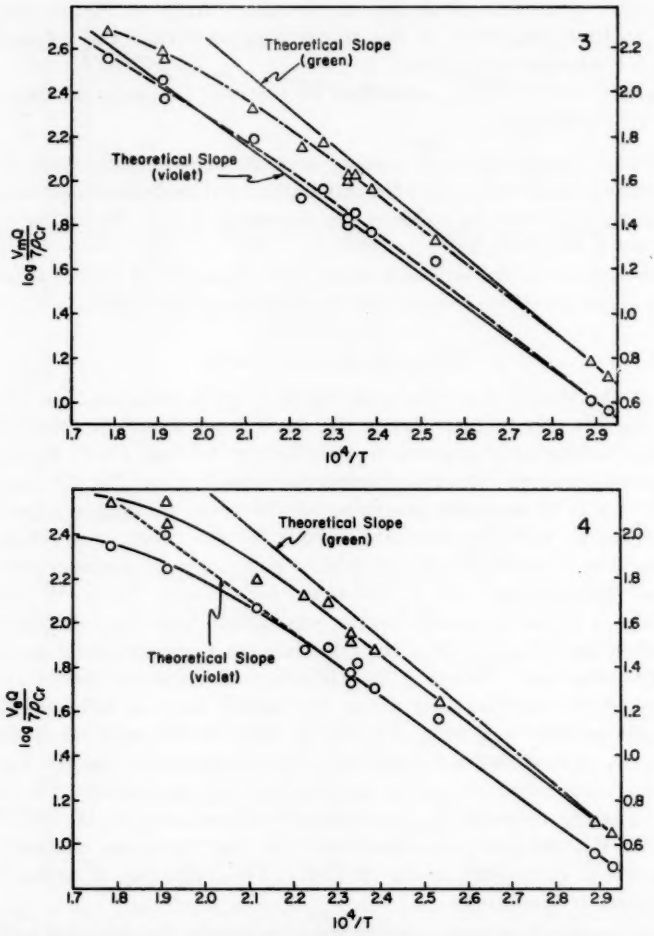

FIG. 3. Plots of $\log[(V_m Q)/(7\rho_{Cr})]$ vs. $10^4/T$ for the violet and green lines. The temperature and density were computed neglecting ionization of the chromium and dissociation of the nitrogen.
FIG. 4. Plots of V_e (at $t = 100$ μsec), similar to Fig. 3.

range in $1/T$. For the upper temperature range, it appears that the correction for ionization has 'overcompensated' the intensity function (Fig. 5 for V_m; a similar curve was obtained for V_e). Note also that the points for the violet line depart more from the theoretical slope than do those for the green lines.

Kinetic data presented below suggest that electron–atom processes control the rate of populating the upper levels and the production of ions. We then tested the possibility that the relaxation time for nitrogen dissociation was appreciably shortened by the presence of Cr^+. These ions, the few negative ions produced by attachment of the electrons, and even the chromium atoms should be highly efficient third bodies for the dissociation reaction. In Fig. 6, the intensities recorded at t_e have been plotted, per eq. [11], against the reciprocal temperatures computed on the assumption of complete

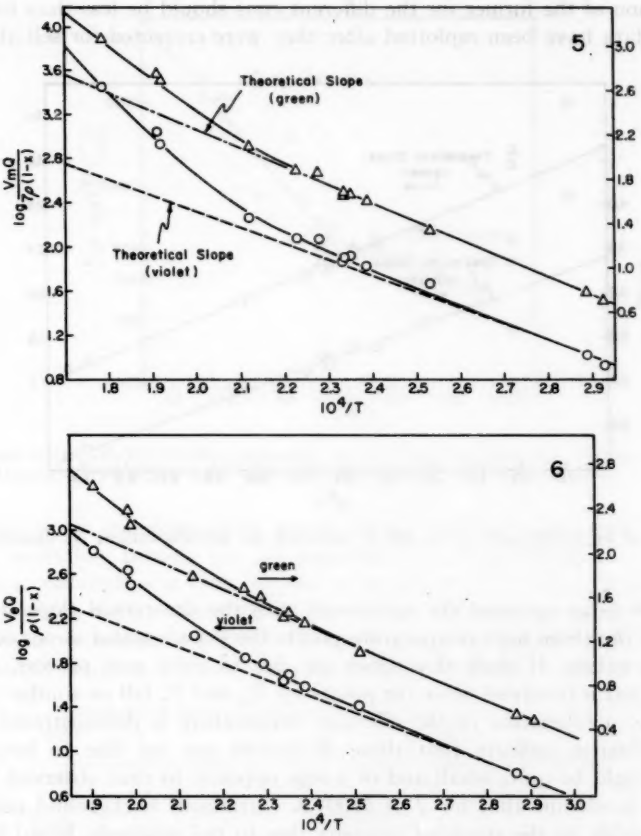

FIG. 5. Plots of $\log[(V_m Q)/7\rho(1-x)]$ vs. $10^4/T$ for the violet and green lines. The temperature and density were computed neglecting the dissociation of nitrogen but allowing for the depletion of chromium by ionization. The fraction ionized is denoted by x.

FIG. 6. Plots of $\log[(V_e Q)/\rho(1-x)]$ vs. $10^4/T$ for the violet and green lines. The temperature and density were computed allowing for the dissociation of N_2 and the ionization of Cr.

equilibrium. Dissociation of the nitrogen, at the highest shock speeds, reduced the computed temperature by $\sim 350°$ K and thereby the magnitude of x. However, the deviations from the theoretical slope were not eliminated due to the concurrent shift of the high-temperature points along the $1/T$ axis with an increase in $(1-x)$.

Except for the points obtained at the highest temperature, the departures from the theoretical slopes may be eliminated by introducing a correction for self-absorption (the term in α, eq. [9]). Since this correction is directly dependent on the population of the lower state, its magnitude for the violet line is proportional to $7\rho_{Cr}(1-x)\,(1-e^{-23,400hc/kT})/Q$ and for the green lines to $5\rho_{Cr}(1-x)e^{-7,593hc/kT}(1-e^{-26,800hc/kT})/Q$. Both functions depend linearly on the density. This is noticeable for the run at $10^4/T = 2.135$ (Fig. 6), which was at a higher density than the adjacent points. In general, the shock densities were lower for the higher-temperature runs. Due to the exp $(-7593hc/kT)$ factor, the green lines should self-absorb approximately 1/33 as much as the violet line, and the

over-all variation of the former for the different runs should be less than for the latter. In Fig. 7, V_e data have been replotted after they were corrected for self-absorption, of

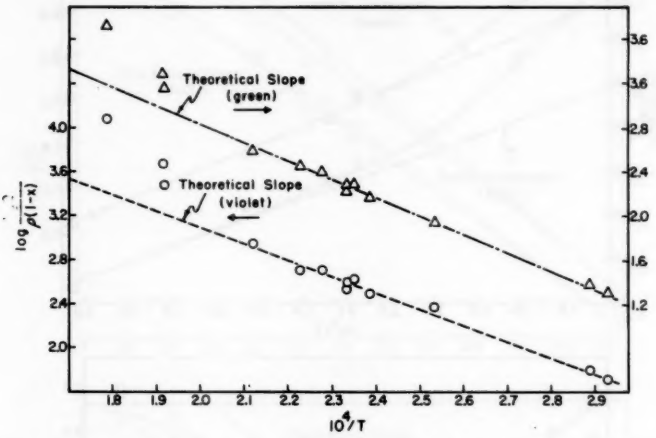

FIG. 7. Plots of $\log[(V_eQ)/\rho(1-x)]$ vs. $10^4/T$ corrected for self-absorption; N_2 dissociation was not included.

such magnitude as to optimize the agreement with the theoretical slope. It is evident that except for the three high-temperature points the experimental slopes coincide with the theoretical values. If these deviations are due to some rate process, a relatively long time constant is involved, since the points for V_m and V_e fall on similar curves; that it is not simply a relaxation in the effective temperature is demonstrated by Fig. 6. Qualitative estimates indicate that these deviations are not due to boundary-layer effects; these would be quite small and of a sign opposite to that observed.* An *ad hoc* explanation is to assume that for $T > 5000°$ K extraneous background radiation contributes appreciably to the recorded intensity due to the relatively broad band passed by the exit slits of the spectrometer.

RISE TIME OF EMISSION

In eq. [16], it is plausible to assume that $(dN_u/dt)_{net} = \rho(1-x)\rho x A \exp(-E_u^*/kT)$ with $(N_u)_e = \rho(1-x)\exp(-E_u/kT)/Q$. This implies that the net rate is proportional to the product of the equilibrium concentration of unionized chromium atoms by the concentration of chromium ions (equal to electrons). A plot of $\log[1/\rho xQV_m(dV/dt)_{\frac{1}{2}}]$ vs. $1/T$ gave a curve with a suggestion of a minimum in the high-temperature region and a large positive slope as $1/T$ increased. Since no reasonable mechanism can be associated with such a temperature dependence, this assumption must be discarded.

A direct second-order dependence of $(dN_u/dt)_{net}$ on the total density leads to a plot of $\log[1/\rho QV_m(dV/dt)_{\frac{1}{2}}]$ vs. $1/T$; Fig. 8 is such a graph for the violet line. The change in slope at $10^4/T = 2.3$ implies that two mechanisms are operative, with $(E_u^* - E_u) =$ 18 kcal mole^{-1} and 54 kcal mole^{-1} for the low- and high-temperature ranges, respectively. (A similar set of values was obtained for the green lines.) For these, we find no acceptable explanation. If the dominant processes for the attainment of the steady-state emission

*Roth and Hartunian (21) analyzed boundary-layer temperatures and densities.

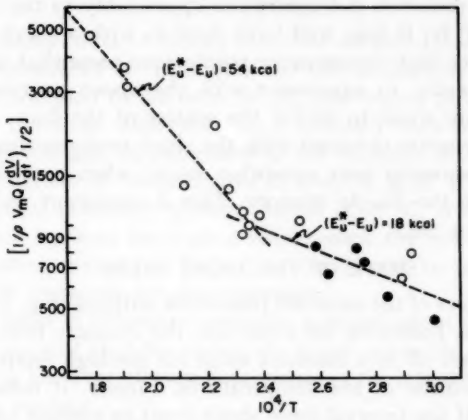

FIG. 8. A plot of $[1/(\rho V_mQ)][(dV/dt)_{\frac{1}{2}}]$, log scale, vs. $10^4/T$ for the *violet* line. The six solid points were taken during the early runs where there was insufficient mixing of $Cr(CO)_6$ and Ar.

involved atom–atom collisions, they would be much slower (18), and the activation energies for excitation, process (*a*), are expected to be much larger.

The simplest assumption is that the rate of excitation, $(dN_u/dt)_{net}$, is dominated by a single process which depends linearly on the density in the same manner as does $(N_u)_e$. This similarity of dependence on density is also imposed on the ionization factor. Graphs of $\log [1/V_mQ(dV/dt)_{\frac{1}{2}}]$ vs. $1/T$ for the violet and green lines are shown in Fig. 9; six

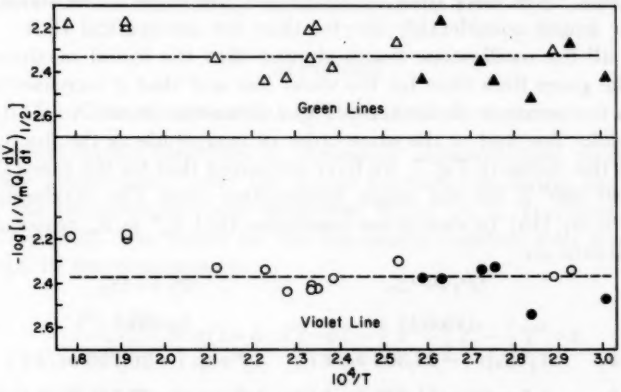

FIG. 9. Plots of $-\log [1/(V_mQ)][(dV/dt)_{\frac{1}{2}}]$ vs. $10^4/T$ for the violet and green lines. The six solid points were taken during the early runs where there was insufficient mixing of the $Cr(CO)_6$ and Ar. The dashed lines have zero slope.

additional points from early runs have been included (full circles and hexagons). Exclusive of the three points for which $1/T < 2\times10^{-4}$, the slopes are nearly zero within the scatter of the data. This is consistent with the postulate that the excitation and ionization of chromium atoms are due mostly to collisions with electrons, for which $E_u^* \approx E_u$ is expected. The initial rise should be exponential in character, as per eq. [14]. Suppose

extraneous background radiation did contribute appreciably to the recorded intensities for runs with $T > 5000°$ K; it may well have done so with a short relaxation time, so that the τ_0's for the three high-temperature points were somewhat shorter than for the others, as observed. Finally, in agreement with the above analysis, the τ_0's for the violet and green lines are equal to within the scatter of the data.

Rather crude measurements obtained with the short response-time circuitry indicate that the rise times for emission were somewhat longer when pure Ar was the ambient medium, compared with the N_2–Ar mixture. This is consistent with the observations of Clouston and Gaydon (8).

SHAPE OF THE LIGHT PULSE

The characteristic shapes of the recorded phototube outputs (Fig. 2) may be accounted for on a qualitative basis. Following the rapid rise, the intensity briefly passes through a slight maximum and levels off to a constant value for the high temperature runs but to a slowly declining magnitude at low temperatures. Finally, it falls rapidly at a time (t_d) which is roughly half the interval from shock front to contact surface, as estimated from ideal shock-tube theory (Table I). It has been established both experimentally (22) and theoretically (23)* that at low initial pressures the flow duration is considerably less than the time computed for ideal shock-tube operation due to effects of the laminar boundary layer behind the shock; low speed gas in the boundary layer near the wall leaks past the contact surface. Quantitative estimates show that this effect is very small for our experiments, even for the lowest initial pressures of 6.3 mm Hg, because of the large diameter of the shock tube. The major source of the short duration flow is the nonideal rupture of the diaphragms, which were comparatively massive (0.032-in. half-hard brass sheets, scored to a depth of 0.016 in., 6 in. in diameter). Under these circumstances, it probably takes more than 10 tube diameters to form the shock, resulting in an effective tube length considerably shorter than the geometrical 18 ft.

Inspection of all the oscilloscope traces showed that the initial maximum was more prominent for the green lines than for the violet line and that it increased in magnitude with rising shock temperature. Reference to Fig. 2 shows that in run No. 51 it is essentially absent for the violet line and of the same order of magnitude as the fluctuation for the green line. From the curves in Fig. 7, we have estimated that for the green line ($V_m - V_e$) corresponds to 40–100° K for the upper temperature runs. The maximum in emission is accounted for by eq. [15]. In view of our conclusion that $E_u^* \approx E_u$, reasonable estimates of the rate constants are

	$^7P_2 \leftarrow {}^7S_3$ (violet)	$^5P_2 \leftarrow {}^5S_2$ (green)
k_j	$A_j^v \exp(-23{,}305\ hc/kT)$	$A_j^g \exp(-26{,}796\ hc/kT)$
k_z	$A_z^v \exp(-31{,}236\ hc/kT)$	$A_z^g \exp(-27{,}745\ hc/kT)$
k_+	ground state: $A_+ \exp(-54{,}541\ hc/kT)$	

Since all the pre-exponential factors should be of comparable magnitude for these similar processes, k_j^v at low temperatures may be large enough to place t_m^v within the rising portion of $(n+c)$. As the temperature is raised, $(n+c)$ rises more rapidly to its final value than do other excitation processes (k_+ has the largest activation energy), and the maxima in intensity become more prominent.

*We acknowledge with thanks permission to refer to the author's paper "On flow duration in low pressure shock tubes" prior to publication.

CONCLUSIONS

The use of dual-channel data to deduce two-color temperatures to the desired precision still awaits the development of techniques for the measurement of intensities with a reproducibility and precision to the order of 2% and with a time resolution of less than a microsecond. For the range 3000–5000° K, if a known trace concentration of $Cr(CO)_6$ is premixed with the test gas, measurement of the intensity of a single Cr emission line does permit the estimation of temperatures to roughly ±50° K, provided care is taken to calibrate the optical–electronic system. Above 5000° K, it appears that extraneous background radiation leads to too high a value, when the intensity is recorded with a spectroscope of modest resolution. Except at the lowest temperatures, the measured intensities had to be corrected for self-absorption.

The rate of excitation of chromium emission is independent of the carbonyl dissociation rate. Our data suggest that the excitation is due primarily to collision of chromium atoms with electrons. The over-all time dependence of the intensity can thus be accounted for in a qualitative manner.

ACKNOWLEDGMENTS

This work was supported by the Air Force (ARDC) under contract AF33(616)-6694. B. E. L. acknowledges with thanks a NATO Research Studentship Grant for the past year. We also thank Mr. Emmett Sutton and Miss Ann Walbran for the use of their program for the computation of equilibrium shock parameters for a dissociating gas and the Ethyl Corporation for the samples of chromium carbonyl used in these experiments. Finally, we gratefully acknowledge the kindness of Dr. R. E. Duff, who computed the thermodynamic functions of $Cr(CO)_6$ (cf. Appendix) and obtained the least-squares fit of coefficients for the free energy expansion via his IBM 704 program.

APPENDIX

Thermodynamic and Partition Functions

A. The thermodynamic functions of chromium carbonyl were recomputed for the ideal gas at one atmosphere, under the simple harmonic-oscillator, rigid-rotor approximation, over the temperature range 300–6000° K (cf. N. J. Hawkins *et al.* J. Chem. Phys. **23**, 2422 (1955)). The values for the free energy function were least-squares fitted to a polynomial in the temperature:

$$\frac{F^\circ_T - H^\circ_0}{RT} = a(1-\ln T) - bT - \frac{c}{2}T^2 - \frac{d}{3}T^3 - \frac{e}{4}T^4 - k.$$

Values for the coefficients were obtained over two ranges.

	Best fit over 300–2000° K	Best fit over 1500–6000° K
k	-3.8958892	$-5.7940996\times10^{+1}$
a	7.5396309	$2.0163532\times10^{+1}$
b	4.2072684×10^{-2}	9.0970966×10^{-3}
c	-3.6287469×10^{-5}	-2.5055972×10^{-6}
d	1.6024045×10^{-8}	3.3908588×10^{-10}
e	-2.7501040×10^{-12}	-1.7854329×10^{-14}

B. The ratio of the partition functions for chromium in the singly ionized and neutral states were computed, to facilitate estimation of the degree of ionization of the metal vapor, via the Saha equation.

T	$10^4/T$	Q_{Cr^I}	$Q_{Cr^{II}}$	$(Q_{Cr^{II}}Q_e)/(Q_{Cr^I})$
3448	2.900	8.07	6.20	1.5366
3704	2.700	8.37	6.29	1.5030
4000	2.500	8.75	6.42	1.4674
4348	2.300	9.25	6.62	1.4313
4762	2.100	9.92	6.93	1.3972
5263	1.900	10.89	7.39	1.3572
5882	1.700	12.38	8.13	1.3134

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LIGHT ABSORPTION STUDIES

PART XVIII. THE ULTRAVIOLET ABSORPTION SPECTRA OF BROMOBENZENES[1]

W. F. FORBES

ABSTRACT

The ultraviolet absorption spectra of bromobenzene in various solvents, and the spectra of a series of substituted bromobenzenes in cyclohexane solution, are reported. The *B*-band spectral data confirm the hypotheses postulated for the *B*-band spectra of chlorobenzenes in the previous part of this series of papers. That is, the bromine atom usually gives rise to greater steric effects than the chlorine atom and the apparent mesomeric interaction as judged from these spectra increases with the size of the halogen substituent.

Both bromobenzene and chlorobenzene *C*-band intensities are unusually small, and this is assumed to account for the frequent *C*-band similarity between the spectrum of a mono-substituted benzene derivative PhX, where X = OH, OCH_3, NH_2, and the spectrum of the corresponding chloro- or bromo-substituted PhX derivative.

INTRODUCTION

Following a study of the ultraviolet absorption spectra of chlorobenzenes (1) and of fluorobenzenes (2), it is intended to extend the discussion of the properties of halogen-substituted benzenes by the study of the spectra of bromobenzenes. It follows from this that the band nomenclature and other terms used in the present paper are identical with the terms as defined or used in the previous papers (1, 2).

THE SPECTRUM OF BROMOBENZENE

The absorption characteristics of bromobenzene in different solvents are listed in Table I.

Table I shows that the location of the *B*-band of bromobenzene is similar for cyclohexane, ether, ethanol, and aqueous solutions. Three points may be noted on comparing the *B*-band of bromobenzene and chlorobenzene. First, a slight wavelength displacement is observed between the *B*-band of bromobenzene in the vapor phase and in cyclohexane solution, whereas no such wavelength displacement was observed for chlorobenzene (1). Since the dipole moment data (3, and references cited therein), infrared data (4), and other data (which are determined by the ground state alone) are similar for chlorobenzene and bromobenzene, it is possible that the electronic excited state of chlorobenzene (which determines predominantly the observed absorption band) is practically independent of the C—Cl dipole but that the C—Br dipole makes some contribution to the relevant electronic excited state of bromobenzene.

Next, the bromobenzene *B*-band usually exists as a maximum accompanied by subsidiary inflections at longer wavelength, and these latter submerged maxima do not facilitate precise comparisons with the locations of absorption bands of other compounds, and in particular with that of chlorobenzene. However, using the absorption band occurring at the longest wavelength, namely the inflection occurring at ca. 222 mμ, or using the "center of gravity" of the bands, the data suggest that the *B*-band of bromobenzene occurs at slightly longer wavelength than the *B*-band of chlorobenzene (cf. ref. 1 and see Fig. 1).

Thirdly, the intensity of the *B*-band of bromobenzene in most solvents is also slightly greater than for the corresponding chlorobenzenes (see, for example, Fig. 1 and cf. also

[1]*Manuscript received November 28, 1960.*
Contribution from the Division of Protein Chemistry, C.S.I.R.O., Parkville N.2 (Melbourne), Victoria, Australia.

TABLE I

Main ultraviolet absorption maxima of bromobenzene in various solvents*

Solvent	*B*-band		*C*-band	
	λ_{max} (mμ)	ϵ_{max}	λ_{max} (mμ)	ϵ_{max}
Vapor phase	209	†	251	†
	213		254	
	218		257	
			260	
			263	
			264	
			266	
Cyclohexane	*211*	*8500*	245	130
	213	9000	252	140
	215	*8500*	*257*	*160*
	ca. *222*	*6000*	259	180
			262	180
			265	200
			272	130
Ether	212	8800	244	130
	215	*8200*	*249*	*120*
	ca. *222*	*5700*	251	135
			255	*155*
			258	160
			261	175
			264	180
			267	*130*
			272	115
Ethanol	212	8100	ca. *245*	*130*
	ca. *215*	*7500*	251	135
	ca. *222*	*5000*	ca. *255*	*155*
			258	160
			261	175
			264	180
			ca. *267*	*130*
			271	110
Water	211	4000	ca. *244*	*100*
	ca. *215*	*4000*	251	100
	ca. *222*	*2500*	255	120
			261	140
			264	130

*Values in italics represent inflections in this and the subsequent table.
†Intensities not determined.

Table I and ref. 1). A simple explanation of these three effects (i.e., the increased solvent–solute interaction, the slight shift to longer wavelength, and the intensification of the *B*-band in bromobenzene compared with these effects in chlorobenzene) is to relate these *B*-band changes to mesomeric interaction as illustrated by resonance structures of type I and to assume that the apparent mesomeric effect is in the order I > Br > Cl

(I)

> F. Since this order of mesomeric interaction is different from that suggested by other data (except for some isolated infrared data (5, 6) which indicate an order of mesomeric interaction parallel to the order as suggested by the ultraviolet data), we refer to it as the *apparent* mesomeric effect of the halogen atoms. Further evidence of the order

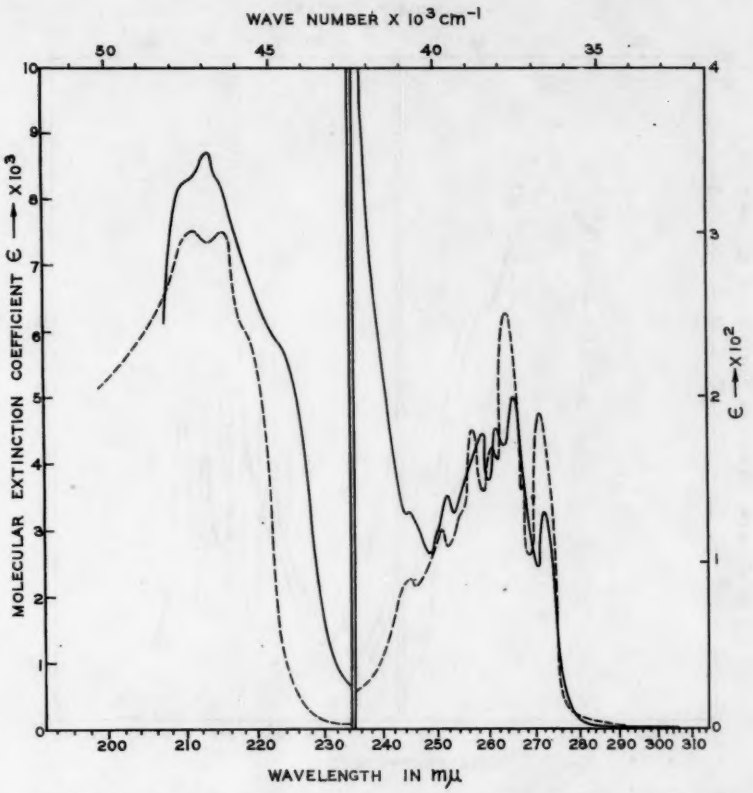


FIG. 1. The ultraviolet absorption spectra in cyclohexane solution of bromobenzene (full line) and chlorobenzene (dotted line).

Br > Cl for the apparent mesomeric effect of the halogen atoms will be presented in the next section.

On the other hand, it should be noted that the majority of ground state data indicate a reverse order of mesomeric interaction, namely F > Cl > Br > I. Moreover, even if the order of mesomeric interaction is as indicated by the present data in the electronically excited state of the observed spectra, it should be pointed out that the order of mesomeric interaction would still be different in these excited levels from the order prevailing in the activated complexes involved in chemical reactions.*

The *C*-bands of bromobenzene and chlorobenzene are similar (see, for example, Fig. 1 and cf. also Table I and ref. 1). Both *C*-bands are unusually weak and this may account for the frequent "overshadowing" of these *C*-bands when another substituent is present, as for example in the spectra of *p*- and *m*-bromofluorobenzenes, which show *C*-bands of comparable intensity to that of fluorobenzene (see Fig. 2).

**A referee has pointed out that ground state calculations have shown that the resonance integrals which measure the π-orbital overlap of two bonded atoms decrease in the order* F > Cl > Br > I *for* =C—X *systems. In support, however, of the arguments presented in this paper, the referee suggests that the excited states for the heavier halogens are more energetically stable and this might tend to reverse the series "in the excited state".*

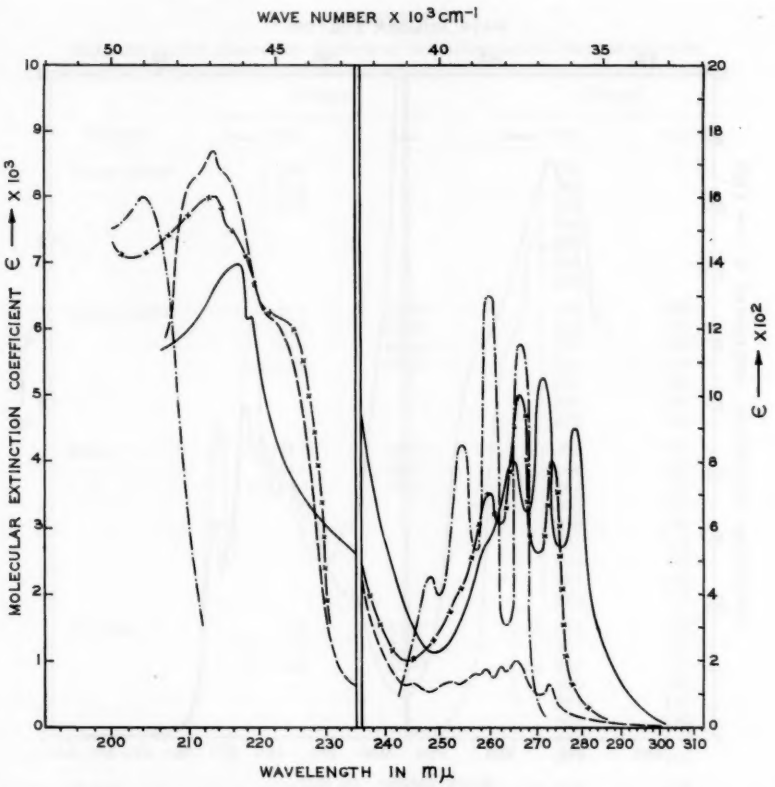
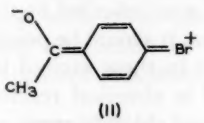


FIG. 2. The ultraviolet absorption spectra in cyclohexane solution of *p*-bromofluorobenzene (——) *m*-bromofluorobenzene (— ×), fluorobenzene (— ·), and bromobenzene (— —).

THE SPECTRA OF PARA-SUBSTITUTED BROMOBENZENES

Using the simplified resonance concept illustrated by structures of type I, electron-withdrawing substituents in the *p*-position of bromobenzene, which permit the extension of the conjugated system as illustrated by resonance structures of type II, would be



(II)

expected to lead to *B*-band absorption at longer wavelength and with increased absorption intensity. In fact, the *B*-band data for *p*-substituted bromobenzenes, where the *p*-substituent is —CHO, —$COCH_3$, or —NO_2, show that the *B*-band now occurs at longer wavelength and with increased absorption intensity (see Table II). Moreover, comparison with the *B*-band data of the related *p*-substituted chlorobenzenes, given in reference 1, shows that the *B*-bands of these *p*-substituted bromobenzenes absorb at slightly longer wavelength and with greater absorption intensity than the corresponding *p*-substituted

TABLE II

Main ultraviolet absorption maxima of substituted bromobenzenes in cyclohexane solution

	Para isomer				Meta isomer						Ortho isomer					
	B-band		*C*-band		Bromobenzene *B*-band		2nd *B*-band		*C*-band		Bromobenzene *B*-band		2nd *B*-band		*C*-band	
Substituent	λ_{max} (mμ)	ϵ_{max}	λ_{max} (mμ)	ϵ_{max}	λ_{max} (mμ)	ϵ_{max}	λ_{max} (mμ)	ϵ_{max}	λ_{max} (mμ)	ϵ_{max}	λ_{max} (mμ)	ϵ_{max}	λ_{max} (mμ)	ϵ_{max}	λ_{max} (mμ)	ϵ_{max}
—F	217	7,000	ca. *259*	*550*	213	8,000*			ca. *253*	*400*	211	9,000*			ca. *255*	*450*
			265	800	ca. *216*	*7,500*			260	700	ca. *215*	*8,500*			260	800
			271	1,050	ca. *225*	*6,000*			266	1,000	ca. *221.5*	*6,000*			266	1,200
			278	900					273	800					272.5	1,200
—Cl	224	13,500	ca. *260*	*250*	ca. *212*	*12,000**			251	120	ca. *212.5*	*13,000**			ca. *250*	*185*
	ca. *230*	*10,000*	267	340	ca. *218*	*10,500**			257	165	ca. *217*	*12,000*			257	170
			274	415	ca. *224*	*7,000*			264	300	ca. *220*	*9,500*			ca. *261*	*175*
			282	335	ca. *228*	*5,500*			271	400	ca. *227*	*6,000*			264	235
									278	325					ca. *268*	*210*
															271	315
															279	275
—Br	*225*	*14,000*	258	310							ca. *214.5*	*12,500**	—	—	ca. *257.5*	*280*
	229	15,000	260	315							219.5	12,500			264.5	260
	ca. *235*	*12,000*	267	365							ca. *223.5*	*11,000*			272	295
			273	*345*											279.5	230
			275	390												
			283	260												
—I	238	17,500	ca. *260*	*1,350*							210	25,000*	230	9,000	253	650
	243	18,000	ca. *265*	*1,150*							ca. *215*	*20,500*	235	10,000	263	600
			ca. *275*	*800*									ca. *258*	*9,500*	269	570
			ca. *285*	*400*											ca. *282*	*350*
$-CH_3$	218	10,400	257	225	ca. *214*	*10,000**			*248*	*130*	ca. *213*	*10,000**			ca. *253*	*195*
	221	10,500	264	315	ca. *225*	*5,500*			*255*	*165*	ca. *224*	*5,500*			ca. *260*	*315*
	227	*7,800*	271	425					262	235					267	460
			278	375					269	320					273.5	440
									275	275					ca. *277.5*	*280*
—OH	224	10,000	ca. *275*	*1,250*	210	7,500*			269	1,450	212.5	8,700*			ca. *268*	*1,750*
			281.5	1,750	ca. *216*	*6,500*			275	2,300					274	2,700
			289	1,500					282	2,250					281	2,650
$-OCH_3$	227	12,500	ca. *277*	*1,300*			ca. *218*	*8,500**	ca. *270*	*1,450*	ca. *218*	*8,300**			ca. *271*	*1,800*
			282	1,750					275.5	2,350	ca. *223*	*7,500*			276.5	2,750
			290	1,550					282.5	2,300					283.5	2,800
$-NH_2$	241	14,000	297	1,750	207	20,000*	237–238	7,800	290	2,400			235	8,500	289	3,000
			ca. *306*	*1,150*					*294*	*1,900*						
$-COCH_3$	253	19,500	*286*	*600*			240	10,000	ca. *282*	*900*			235	4,900	282	700
							ca. *245–246*	*8,000*	287	1,100						
									296	950						
—CHO	258.5	22,000	ca. *277*	*2,350*	212.5	20,000*	243.5	7,000	292	825	210	27,000*	246	12,500	ca. *287*	*1,500*
	264	19,000	290	1,250	218	18,000			302	700			ca. *252*	*10,000*	295	2,100
															304	1,750
$-NO_2$	270	13,000	ca. *285*	*9,000*	210	18,000*	254	7,500	293	1,300	211	15,000*	ca. *246*	*3,700*	293	1,300
			ca. *295*	*4,500*	214.5	18,500			301	1,300	ca. *218*	*13,500*				
					ca. *223*	*16,500*			ca. *313*	*750*						

*B-band assignment doubtful.

chlorobenzenes (cf. also refs. 7, 8 for the *B*-bands of the *p*-chloro and *p*-bromo derivatives of nitrobenzenes and acetanilides). This indicates that the bromine atom has a greater ability to release electrons in the electronic excited state than the chlorine atom and again confirms the order of apparent mesomeric interaction Br > Cl for the halogen atoms as suggested in the previous section. It is also worth noting that the reported dipole moment of compounds like *p*-bromonitrobenzene is slightly greater than that of *p*-chloronitrobenzene (3) although dipole moment data are determined only by the ground state.

A plot of the wavelength displacements (in cm^{-1} units) for four *p*-substituted bromobenzenes, where the *p*-substituent was —H, —CHO, —$COCH_3$, or —NO_2, against various functions such as σ^*, $(\sigma_p - \sigma')$ values for the above substituents did not give rise to a straight line. Nor was a straight line obtained when the wavelength displacements in cm^{-1} units for *p*-substituted acetophenones, when the *p*-substituent was H, F, Cl, Br, or I, were plotted against the σ^* or $(\sigma_p - \sigma')$ values of these substituents. However, as noted by Schubert and co-workers (9), a graph relating ν_{max} values of the *B*-band in the gas phase against molar refraction values does afford an approximately linear relationship for the *p*-halonitrobenzenes, *p*-haloacetophenones, and *p*-haloanisoles, in this way confirming the relationship between molecular polarizability (as measured by molar refraction values) and certain absorption spectra.

Table II not only includes the *p*-substituted bromobenzenes discussed in the previous paragraph, but also includes *p*-substituted bromobenzenes where the *p*-substituent does not extend the conjugation in the most probable resonance form. These spectra, as expected from simple resonance theory, involve so-called locally excited states; that is, these spectral data (which are listed at the beginning of Table II) indicate that the *B*-bands of these compounds usually resemble the *B*-band of one of the monosubstituted parent compounds. Of the two substituents, normally the substituent with the greater electron-donating power will predominantly determine the wavelength and intensity of the monosubstituted benzene *B*-band, while the other substituent will exert only a "secondary" effect on the *B*-band. For example, the *B*-band of *p*-bromoiodobenzene in cyclohexane solution shows the characteristic *B*-band doublet near 240 mμ fairly close to the *B*-band absorption doublet of iodobenzene, near 230 mμ, and the spectrum of *p*-bromoaniline resembles that of aniline. In both these examples the corresponding *C*-bands resemble the *C*-bands of iodobenzene and aniline respectively. This is further illustrated by Fig. 3, which shows the similarity of the spectra of *p*-bromoaniline and aniline and the dissimilarity from the absorption spectrum of bromobenzene.

For other compounds the assignments are more doubtful. For example, the *B*-band of *p*-bromofluorobenzene in cyclohexane solution resembles the band of bromobenzene more than the *B*-band of fluorobenzene (see Fig. 2) since the latter band occurs below 205 mμ (2); however, the *C*-band of *p*-bromofluorobenzene resembles the *C*-band of fluorobenzene more than the *C*-band of bromobenzene. Next, the *B*-bands of *p*-bromophenol, *p*-bromoanisole, and *p*-chlorobromobenzene are tentatively ascribed to phenol, anisole, and bromobenzene absorption respectively with the *p*-bromo or *p*-chloro substituents facilitating the transition by means of a secondary interaction (cf. also ref. 1). Partial support for these assignments is provided by the lack of fine structure in the *B*-bands of *p*-bromophenol and *p*-bromoanisole, and by the relevant *C*-bands (see Table II), which may readily be identified as phenol or anisole *C*-bands (see also Fig. 4, a fact that illustrates the similarity of the spectra of *p*-bromoanisole and anisole and the dissimilarity from the absorption spectrum of bromobenzene). On the other hand, *C*-band

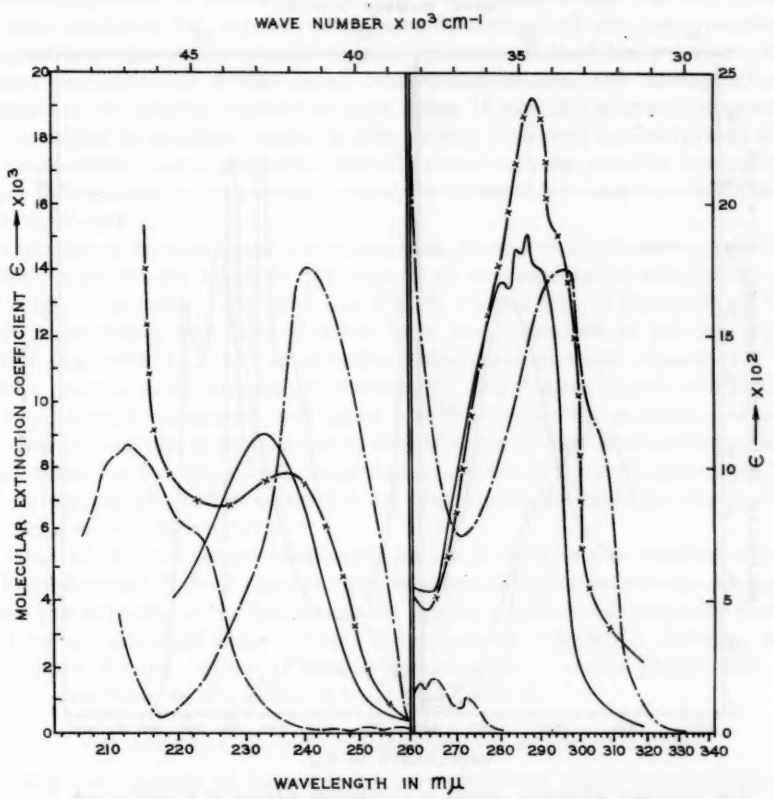


FIG. 3. The ultraviolet absorption spectra in cyclohexane solution of aniline (——), *p*-bromoaniline (—·—), *m*-bromoaniline (—×—), and bromobenzene (— —).

similarities can be misleading (see the preceding discussion of the spectra of *p*-bromofluorobenzene) and also some of the solvent changes suggest a different assignment. For example, the wavelength displacement observed on determining the *B*-band of *p*-bromoanisole in the vapor phase and in an inert solvent medium (2.8 mμ (9); 550 cm^{-1}) is closer to the wavelength displacements observed under similar conditions for bromobenzene (ca. 2 mμ (see Table I); 440 cm^{-1}) than to that observed for anisole (4.6 mμ (9); 980 cm^{-1}).

A *p*-bromo substituent generally appears to cause the *B*-band absorption to occur at longer wavelength even if both substituents are electron-donating as in *p*-dibromobenzene; the wavelength displacement relative to the non-bromo-substituted reference compound is usually not less than ca. 10 mμ and the absorption intensity is appreciably enhanced (see Table II and previous parts of this series of papers). This displacement may be visualized as the bromine atom donating electrons in the electronic excited state and this effect of the *p*-bromo substituent is again slightly larger than the previously noted similar effect of a *p*-chloro substituent (1).

Concerning *C*-band absorption it may be noted that a *p*-bromo substituent generally (except in examples where resonance structures of type II may be assumed to be involved)

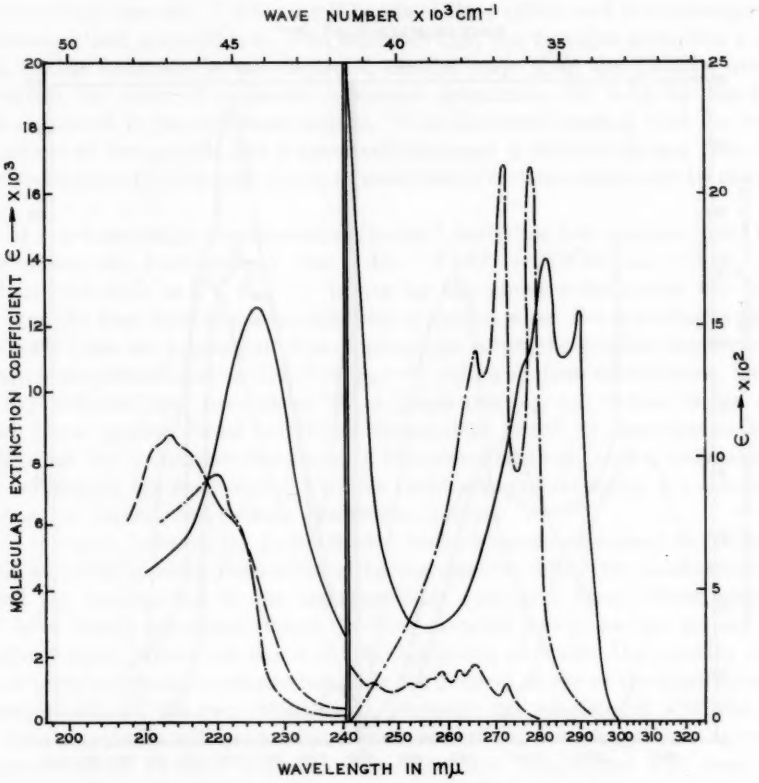


FIG. 4. The ultraviolet absorption spectra in cyclohexane solution of *p*-bromoanisole (——), anisole (—·), and bromobenzene (— —).

does not appear to affect the *C*-band structure of a number of *p*-substituted bromobenzenes; that is, the *C*-bands of these *p*-substituted bromobenzenes may occur at slightly longer wavelength and with slightly decreased absorption intensity but are otherwise similar to the *C*-bands of the corresponding non-bromo-substituted parent compounds (see, for example, Figs. 2, 3, and 4 and cf. ref. 10). The absence of any marked evidence of bromobenzene *C*-band absorption on the structure of the *C*-band may again be related to the low-intensity *C*-band absorption in bromobenzene. That is, the bromobenzene *C*-band absorption is normally hidden by other more intense *C*-band absorption except in compounds like *p*-bromochlorobenzene where both constituent *C*-bands, namely the *C*-bands of chlorobenzene and bromobenzene, are weak.

THE SPECTRA OF META-SUBSTITUTED BROMOBENZENES

For *m*-substituted bromobenzenes, a *B*-band associated with the bromobenzene *B*-band can usually be ascertained; the *C*-band only rarely corresponds to bromobenzene *C*-band absorption (cf. last paragraph of previous section). For example, for *m*-bromofluorobenzene the *B*-band absorption at 213 mμ is associated, at least partly, with bromobenzene *B*-band absorption (see Tables I and II), whereas the *C*-band absorption of

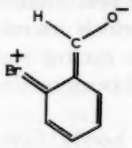
m-bromofluorobenzene is similar to that of fluorobenzene (see Fig. 2). Frequently the *C*-band similarity for *m*-substituted bromobenzenes and the corresponding non-bromo-substituted parent compounds is more pronounced than the previously referred to *C*-band similarity for *p*-substituted bromobenzenes and the corresponding non-bromo-substituted benzene derivatives (see Table II and the relevant reference compounds described in previous papers of this series). This may be interpreted in terms of resonance theory since electronic perturbations, such as may be associated with resonance interactions in the *p*-isomer, would be of less importance in a *m*-disubstituted benzene derivative.

For a number of *m*-substituted bromobenzenes, instead of the bromobenzene *B*-band, a "second" *B*-band—for example, the *B*-band of *m*-bromoaniline associated with the aniline *B*-band—is readily identified (see Fig. 3; reasons why *m*-disubstituted benzene derivatives sometimes show two *B*-bands have been discussed in previous papers of this series (e.g. refs. 1, 2)). The *m*-bromo substituent generally causes this *B*-band absorption to occur at slightly longer wavelength. This displacement is less pronounced than for a *p*-bromo substituent and about equally effective for *m*-chloro and *m*-bromo substituents. It may be noted that both *m*-chloro and *m*-bromo substituents have the effect of enhancing the integrated intensities of the O—H infrared absorption band in phenol (integrated absorption intensities are well known to relate to mesomeric interactions) to a similar extent (11).

The effect of the non-bromo substituent on the *location* of the maximal wavelength of the bromobenzene *B*-band absorption in *m*-substituted bromobenzenes appears to be small but the intensity of the bromobenzene *B*-band is sometimes enhanced (see Tables I and II for the relatively more intense "bromobenzene *B*-bands"). However, since the bromobenzene *B*-band occurs at shorter wavelength, it seems likely that *A*-band transitions contribute to the observed band (see Table II).

THE SPECTRA OF ORTHO-SUBSTITUTED BROMOBENZENES

The ultraviolet spectra of *o*-substituted bromobenzenes would be expected to show the effects of appreciable steric interactions. In fact, the second *B*-band (that is, the *B*-band *not* associated with bromobenzene absorption) of an *o*-bromo-substituted compound frequently occurs with decreased absorption intensity compared with the second *B*-band of the corresponding *o*-chloro-substituted compound.* (See Table II and ref. 1;



(III)

cf. also the data reported for nitrobenzenes (7), acetanilides (8), N,N-dimethylanilines (12), and 5-phenyltetrazoles (13), which show that the maximal extinction coefficient

**An example of an exception is the B-band of o-bromobenzaldehyde (see Table II) which is more intense than the corresponding B-band of o-chlorobenzaldehyde (1; cf. also the ε values reported (19) for o-chloro- and o-bromophenyl-2,5-diphenyloxazoles). This may be because in o-bromobenzaldehyde and related compounds the effect of mesomeric interactions, as illustrated by resonance forms of type III, is still appreciable and hence the greater apparent mesomeric effect of the bromo substituent leads to increased absorption intensity. For most other o-substituted bromobenzenes resonance structures of type III may be of little importance because the non-planarity of the molecule inhibits the necessary conjugation.*

of the *B*-band of the *o*-bromo derivative is less than the corresponding maximal extinction coefficient of the *o*-chloro derivative.) Moreover, in examples like *o*-bromoacetophenone, where the non-bromo substituent is large, the absorption intensity is also smaller than the intensity of the corresponding *m*-isomer (see Table II). This decreased extinction coefficient, relative to the *m*-isomer, is also accompanied by a hypsochromic wavelength displacement, which in this type of compound is assumed to be associated with steric interactions. Since non-planarity in *o*-bromo-substituted benzene derivatives is also suggested from other data (see, for example, refs. 3, 14, 15, 16, 17, 18), the above spectral data are assumed to indicate that the bromine atom, because of its larger size relative to the chlorine atom (cf. ref. 14), more effectively dislodges the non-bromo substituent from the plane of the benzene ring and in this way gives rise to the observed spectral changes.

However, the ultraviolet data do not consistently indicate that the bromo substituent exerts a greater steric effect than the chloro substituent (see footnote of previous paragraph). A similar lack of consistency is found when comparing the ϵ values of *o*-bromo-substituted benzene derivatives with the ϵ values of *o*-methyl-substituted benzene derivatives. For example, for acetanilides (8) the *o*-bromo derivative shows more intense *B*-band absorption than the *o*-methyl derivative (see also reference 20 for the spectra of the corresponding benzoic acids). However, more frequently, for example, for anilines (21), or benzaldehydes (22), the *o*-methyl derivative shows more intense *B*-band absorption than the *o*-bromo derivative (see also refs. 19, 23). The data thus indicate that the effective interference radius of the bromine atom is greater than that of the methyl group (or than that of the chlorine atom; cf. refs. 14, 15), but it remains to provide an explanation for the minority of anomalous data. This can be done by considering dipole moments. That is, a methyl substituent, which has a dipole moment in the opposite direction to that of the halogen atoms, may cause a considerably different over-all dipole moment and in this way compounds like *o*-toluic and *o*-bromobenzoic acids, for example, may possess appreciably different dipole moments. Moreover, steric interactions (which are assumed to be present) may increase the over-all dipole moment of one molecule while decreasing the dipole of another, similar molecule (cf. ref. 3, where it was pointed out for nitrobenzenes that an *o*-methyl substituent increases the dipole moment by increasing the angle between the C—N and C—Me bonds, whereas an *o*-bromo or *o*-chloro substituent may decrease the over-all dipole moment). Since absorption intensities are determined by transition probabilities which, in turn among other things, are governed by transition moments—which are related to the change in dipole moment on passing from the ground state to the excited state—it is evident that the absorption intensities of the above *o*-disubstituted benzene derivatives are not determined merely by steric interactions.

Although intramolecular hydrogen bonds have been postulated in *o*-substituted bromobenzenes because of evidence from other physical data (24, 25, 26), as for the previously discussed spectra of *o*-substituted chlorobenzenes or fluorobenzenes (1, 2), there is little evidence *from the above ultraviolet spectra* for such a hydrogen bond. That is, no unusual wavelength displacement or unusual change in ϵ_{max} values, such as may be ascribed to the presence of an intramolecular hydrogen bond, is observed in the spectrum of *o*-bromophenol compared with the spectrum of *m*-bromophenol or *o*-bromoanisole (see Table II); only for *o*-bromoaniline small intensity increases are observed in the ϵ_{max} values compared with the ϵ_{max} values of the *m*-isomer, but it is not certain that these intensity increases are caused by an intramolecular hydrogen bond.

The *C*-bands in *o*-substituted bromobenzenes usually occur with increased absorption intensity compared with the *C*-bands of the corresponding *m*-isomers, except in compounds, such as *o*-bromoacetophenone, which are believed to be appreciably non-planar because of steric interactions. For a number of *o*-chloro- and *o*-bromo-substituted compounds the *C*-bands are remarkably similar; for example, *o*-chloroaniline, λ_{max} 291 mμ, $\epsilon = 2900$ and *o*-bromoaniline, λ_{max} 289 mμ, $\epsilon = 3000$ (see ref. 1 and Table II). This *C*-band similarity has previously been discussed (see Introduction).

EXPERIMENTAL

The ultraviolet absorption spectra were determined by standard methods in 1-cm cells using either a Beckman DU or DK 2 spectrophotometer or Unicam SP 500 spectrophotometers calibrated against a didymium filter. Wavelength readings obtained on the Unicam and Beckman instruments were found to be within 1 mμ of each other. The accuracy of λ_{max} values is estimated to be ± 1 mμ, and the precision of ϵ_{max} values $\pm 5\%$, or better. Values of ϵ_{max} were reproducible in most cases to $\pm 2\%$. For each compound at least two independent sets of observations were made. The ultraviolet maxima are listed in Tables I and II. Some of the spectra have previously been described in this series and elsewhere. Whenever possible, the bromobenzene spectra were determined under conditions identical with those under which previous spectra in this series had been determined. Frequently, but not always, the data were in good agreement with recent values reported by other workers.

The bromobenzenes were mostly commercial materials, purified by distillation or recrystallization until their boiling points and refractive indices or melting points showed them to be sufficiently pure. The solvents used were spectroanalyzed cyclohexane (Fisher) and other commercially available spectroanalyzed solvents.

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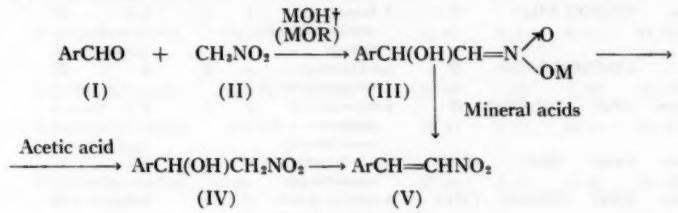
NOTES

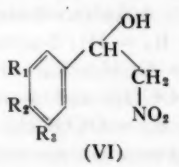
A NOTE ON THE PREPARATION OF SOME 1-PHENYL-2-NITROETHANOL DERIVATIVES

R. A. Heacock, O. Hutzinger, and C. Nerenberg*

It has been known for many years that aromatic aldehydes (I) and nitromethane (II) will undergo a condensation reaction, under alkaline conditions, to yield the alkali metal derivatives of the *aci*-form of the monohydric nitroalcohols (III). The free nitroalcohols (IV) can be liberated on acidification of the alkali metal derivative with acetic acid; however, the products will often dehydrate with extreme ease to form the corresponding ω-nitrostyrenes (V), unless certain precautions are taken (cf. ref. 1).



In this laboratory, we have been primarily interested in obtaining certain alkyl, acyl, and aroyl derivatives of 1-(3,4-dihydroxyphenyl)-2-nitroethanol and 1-(3,4,5-trihydroxyphenyl)-2-nitroethanol. A few of these nitroalcohols had previously been described in the literature namely: 3,4-methylenedioxy-α-nitromethylbenzyl alcohol (VI: R_1+R_2 = OCH_2O; R_3 = H) (2, 3, 4, 5); 3,4-diacetoxy-α-nitromethylbenzyl alcohol (VI: R_1 = R_2 = $OCOCH_3$; R_3 = H) (6); and 4-benzyloxy-3-methoxy-α-nitromethylbenzyl alcohol (VI: R_1 = OCH_3; R_2 = $OCH_2C_6H_5$; R_3 = H) (7). The 3,4-diacetoxy derivative was obtained using aqueous sodium bicarbonate as the condensing agent (6); however, the other nitroalcohols were prepared either using sodium methoxide under anhydrous conditions or aqueous alcoholic potassium hydroxide to effect the condensation (1, 2, 3, 4, 5, 7).



(VI)

Some of the aforementioned procedures are rather tedious to carry out and this note reports a rapid and simple method for preparing nitroalcohols of this general type in good yield. The procedure involves the condensation of a suitable aldehyde with excess nitromethane at about 5° in the presence of sodium hydroxide in aqueous alcoholic solution. The optimum reaction time, which was of very short duration (⩾ 3 minutes), varied from case to case and was determined empirically.

As well as the nitroalcohols mentioned above, the following new 1-phenyl-2-nitroethanol

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†M = Na *or* K.

TAB

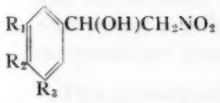
Preparation of nitroalcohols

Nitroalcohol prepared				Reagents used and reaction conditions						
Name	R_1	R_2	R_3	Aldehyde*		(g)	CH_3NO_2 (ml)	95% C_2H_5OH (ml)	10% NaOH aq. (ml)	2% AcOH (ml)
3,4-Methylenedioxy-α-nitromethylbenzyl alcohol	OCH_2O		H	Piperonaldehyde	*a*	10	11	135	20	160
4-Acetoxy-3-methoxy-α-nitromethylbenzyl alcohol	CH_3O	CH_3COO	H	4-Acetoxy-3-methoxybenzaldehyde	*b*	5	3.5	35	10	80
3-Acetoxy-4-methoxy-α-nitromethylbenzyl alcohol	CH_3COO	CH_3O	H	3-Acetoxy-4-methoxybenzaldehyde	*b*	5	3.5	50	10	80
3,4-Diacetoxy-α-nitromethylbenzyl alcohol	CH_3COO	CH_3COO	H	3,4-Diacetoxybenzaldehyde	*c*	5	3	25	10	90
4-Benzyloxy-3-methoxy-α-nitromethylbenzyl alcohol	CH_3O	$C_6H_5CH_2O$	H	4-Benzyloxy-3-methoxybenzaldehyde	*d*	1	0.7	7	1.55	13
3,4,5-Trimethoxy-α-nitromethylbenzyl alcohol	CH_3O	CH_3O	CH_3O	3,4,5-Trimethoxybenzaldehyde	*e*	3	2.1	21	5.55	48
4-Acetoxy-3,5-dimethoxy-α-nitromethylbenzyl alcohol	CH_3O	CH_3COO	CH_3O	4-Acetoxy-3,5-dimethoxybenzaldehyde	*f*	1	0.7	18	1.65	13.5

*Sources of aldehydes: *a* = Eastman Kodak. *b* = Prepared by the acetylation of vanillin and isovanillin respectively by an adaptation of the method described by Kanao for the preparation of 3,4-diacetoxybenzaldehyde (6). *c* = Prepared by the method of Kanao (6). *d* = 4-Benzyloxy-3-methoxybenzaldehyde (m.p. 64°) was prepared from vanillin by benzylation with benzyl chloride in the presence of aqueous potassium hydroxide. This substance has previously been prepared by alternative routes and the melting point is quoted as 63–64° (9). *e* = Aldrich Chemical Company. *f* = Prepared by the acetylation of Syringaldehyde

derivatives have been prepared: 4-acetoxy-3-methoxy-α-nitromethylbenzyl alcohol (VI: $R_1 = OCH_3$; $R_2 = OCOCH_3$; $R_3 = H$); 3-acetoxy-4-methoxy-α-nitromethylbenzyl alcohol (VI: $R_1 = OCOCH_3$; $R_2 = OCH_3$; $R_3 = H$); 3,4,5-trimethoxy-α-nitromethylbenzyl alcohol ($R_1 = R_2 = R_3 = OCH_3$) and 4-acetoxy-3,5-dimethoxy-α-nitromethylbenzyl alcohol ($R_1 = R_3 = OCH_3$; $R_2 = OCOCH_3$). Attempts to prepare a number of related nitroalcohols by this method were not successful: (i) veratraldehyde gave mainly 3,4-dimethoxy-ω-nitrostyrene, even after cooling the reaction mixture below 0°; (ii) protocatechualdehyde, syringaldehyde, vanillin, and isovanillin did not react with nitromethane under the above conditions. This is not altogether surprising in view of the fact that three of these aldehydes have a para hydroxyl group, which has been reported by several workers to hinder this type of condensation (1, 8); (iii) 3,4-dibenzoyloxybenzaldehyde, 3-benzyloxy-4-methoxybenzaldehyde, 3,4-diethoxycarbonyloxybenzaldehyde, and 3-ethoxycarbonyloxy-4-methoxybenzaldehyde gave oily products, for which satisfactory analyses could not be obtained (for the nitroalcohol) even after distillation in high vacuum. It would appear in these cases that condensation had occurred, but partial decomposition of the products took place in the isolation or distillation stages. The infrared spectra of thin films of these oils showed strong absorption in the 2.79 to 2.81 μ region, probably due to the stretching vibrations of the benzyl alcohol hydroxyl group. (The authenticated nitroalcohols all exhibited marked absorption in this region (2.75 to 2.88 μ).) Recently Axelrod, Senoh, and Witkop have reported the OH stretching fre-

LE I



Reaction time (sec)	Yield of purified product (g)	Properties of products: Crystalline form	m.p. (°C)	Literature m.p. (°C) (if known)	Analysis: Found C	Found H	Found N	Calculated C	Calculated H	Calculated N
60	9.3	Fine colorless needles from benzene	95	91(5),‡ 95–96(4), 91(3), 94(2)	51.34	4.41	6.61	51.19	4.30	6.63
45	5.5	Fine colorless needles from benzene	90.5		51.90	5.12	5.59	51.77	5.14	5.49
30	4	Fine colorless needles from benzene	103–104		51.87	5.21	5.43	51.77	5.14	5.49
30	4.9	Colorless plates from ethanol	153–155	155(6)	50.99	4.59	5.02	50.88	4.63	4.95
45	0.65†	Colorless small prisms from benzene	107–108.5	107–109(7)	63.44	5.71	4.13	63.36	5.65	4.62
45	2.4	Colorless fine needles from benzene	109		51.60	5.84	5.33	51.36	5.88	5.45
20	1	Fine colorless needles from ethanol	200		50.74	5.39	5.07	50.52	5.30	4.91

(Aldrich Chemical Company) by an adaptation of the method described by Kanao for the preparation of 3,4-diacetoxybenzaldehyde (6), m.p. 115°. Freudenberg and Hübner prepared this compound by a different route and quote m.p. 114° (10).

†The crude product, a yellow oil, was stirred with concentrated sodium bisulphite solution for 20 minutes before recrystallization from benzene.

‡The melting point has been incorrectly reported as 98–99° in Chem. Abstr. **48**, 13,653 (1954).

quency of 4-benzyloxy-3-methoxy-α-nitromethylbenzyl alcohol as 2.81 μ in chloroform solution (7).

In a few cases, the corresponding ω-nitrostyrenes had not been previously described in the literature and these derivatives were prepared in good yield, from the nitroalcohol in question, by an adaptation of the method described by Kanao for the preparation of 3,4-diacetoxy-ω-nitrostyrene from 3,4-diacetoxy-α-nitromethylbenzyl alcohol (6).

EXPERIMENTAL

*General Procedures**

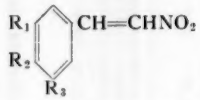
(*i*) *Nitroalcohols.*—Aqueous sodium hydroxide (10%; 1.05 mole) was added with vigorous stirring to a mixture of the aldehyde (1.0 mole) and nitromethane (2–3 mole) dissolved in 95% ethanol at ca. 5°; the reaction mixture was vigorously stirred for a short time (⩾3 minutes), accurately timed on a stop watch. Aqueous acetic acid (2%) was added to arrest the reaction and decompose the sodium derivative of the nitroalcohol; the crude product precipitated out as a yellow or colorless solid or oil. After being allowed to stand at 4° for 4–5 hours, the crude product was filtered and purified by repeated recrystallization from a suitable solvent.

(*ii*) *ω-Nitrostyrenes.*—A suspension of the nitroalcohol and the same weight of fused

**The specific quantities of reagents used and reaction times employed are given in Tables I and II.*

TABLE II

Preparation of ω-nitrostyrenes



ω-Nitrostyrene prepared				Amount of nitro-alcohol used in g	Properties of product			Analysis					
					Yield of purified product (g)	Crystalline form (from 95% ethanol)	m.p. (°C)	Found			Calculated		
Name	R_1	R_2	R_3					C	H	N	C	H	N
4-Acetoxy-3-methoxy-ω-nitrostyrene*	CH_3O	CH_3COO	H	2	1.5	Fine yellow needles	166–167	55.62	4.62	5.90	55.69	4.68	5.91
3-Acetoxy-4-methoxy-ω-nitrostyrene	CH_3COO	CH_3O	H	0.85	0.7	Yellow needles	142	55.51	4.61	5.75	55.69	4.68	5.91
4-Acetoxy-3,5-dimethoxy-ω-nitrostyrene	CH_3O	CH_3COO	CH_3O	0.3	0.25	Pale yellow plates	177	54.01	4.87	5.21	53.93	4.90	5.24
3-Ethoxycarbonyloxy-4-methoxy-ω-nitrostyrene	C_2H_5OCOO	CH_3O	H	†	—	Fine yellow needles	116	54.13	4.98	5.22	53.93	4.90	5.24

*The preparation of this substance has been reported previously by Kobayashi; however, the original Japanese paper is not available to the authors and no preparative details or melting points are given in Chemical Abstracts, but according to Beilstein, the substance was obtained by acetylation of 4-hydroxy-3-methoxy-ω-nitrostyrene and had m.p. 161–162° (11).
†A small quantity of the crude reaction mixture from the attempted preparation of 3-ethoxycarbonyloxy-4-methoxy-α-nitromethylbenzyl alcohol was used.

sodium acetate in acetic anhydride (five times the weight of one of the other reagents) was boiled under reflux for 10 minutes. After cooling, the reaction mixture was poured into water; a yellow solid separated out, which was filtered off, washed with water, and recrystallized from 95% ethanol.

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PREPARATION AND DEHYDRATION OF DI-(1-HYDROXYCYCLOALKYL)-ALKANES[1]

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1-Alkylcycloalkanols have been employed (1, 2) as intermediates in the extension of monofunctional aliphatic chains. An obvious extension of this synthetic method would be the use of di-(1-hydroxycycloalkyl) compounds in chain extension of α,ω-dicarboxylic acids of the aliphatic series. Although there is no record (3) of the use of such intermediates for this purpose, diols of the requisite structural type have been made in isolated previous investigations. We have now prepared diols having five-, six-, seven-, and eight-membered rings and with 2 to 10 methylene groups connecting them, using procedures already described. The results are summarized in Table I.

Method A, reaction of a diester with two moles of a Grignard reagent from a dihalide, has been studied previously only in the synthesis of 1,1′-ethylenedicyclopentanol and 1,1′-ethylenedicyclohexanol from ethyl succinate and respectively tetramethylene dibromide and pentamethylene dibromide (4). We attempted to use hexamethylene dibromide and 1,3-dibromo-2,2-dimethylpropane in similar syntheses but in each case obtained no crystalline product but the major outcome was evidently a polymer. Diethyl 2,2-dimethylglutarate was successfully condensed with 2 moles of the derived Grignard reagent from pentamethylene dibromide although one might have expected the gem dimethyl group to hinder or deactivate one of the ester groups sterically.

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TABLE I

Compound	M.p., °C	Method of prep.	Yield,* %	Lit. m.p.	Ref.	Analysis C Found	Analysis C Calc.	Analysis H Found	Analysis H Calc.	Dehydration product B.p.	Dehydration product *n*	Dehydration product H. equiv.¶
1,1′-Ethylenedicyclopentanol	134	A	65	131.2–1.4	12					85–9°/ 0.5 mm	1.4810 (21°)	27, 21%
		C	48	135	4							
1,1′-Ethylenedicyclohexanol	130–1	A	46	128–9	9					90–7°/ 0.5 mm	1.4821 (20°)	28, 33%
		C	42									
1,1′-Ethylenedicycloheptanol	123–4	C	16	Oil	10†	75.6	75.6	11.7	11.8			
1,1′-Ethylenedicyclooctanol	123–4	C	18			76.4	76.6	12.0	12.0			
1,1′-Trimethylenedicyclopentanol	119–21	A	58			73.4	73.6	11.4	11.3			
1,1′-Trimethylenedicyclohexanol	122–3	A	69	120	25					111–5°/ 14 mm	1.4916 (25°)	97%
1,1′-Tetramethylenedicyclopentanol	98–9	B	49	91.8–2.5	14‡							
		D	68									
1,1′-Tetramethylenedicyclohexanol	113.5–5	B	53	103	26					157–9°/ 15 mm	1.5104 (25°)	101%
		D	71									
1,1′-Tetramethylenedicycloheptanol	85–6	D	65			76.3	76.6	12.1	12.0			
1,1′-Pentamethylenedicyclopentanol	63.5–4	B	83			75.1	75.0	11.7	11.7	101–4°/ 0.5 mm	1.5103 (25°)	94%
1,1′-Pentamethylenedicyclohexanol	72–3	B	76			76.4	76.2	11.8	11.9			
1,1′-Hexamethylenedicyclopentanol	87–9	B	86			75.8	75.6	11.9	11.8	109–14°/ 0.5 mm	1.5092 (25°)	96%
1,1′-Hexamethylenedicyclohexanol	83–4	B	71			76.3	76.6	12.1	12.0			
1,1′-Octamethylenedicyclopentanol	83–4	A	52			76.4	76.6	11.8	12.0	141–5°/ 0.8 mm	1.4811 (25°)§	94%
1,1′-Octamethylenedicyclohexanol	87.5–8	A	31			77.4	77.5	12.2	12.3	157–61°/ 0.4 mm	1.4911 (25°)‖	96%
1,1′-Decamethylenedicyclohexanol	56–7	B	38			77.9	78.1	12.2	12.4			
$HO.C_6H_{10}.CH_2.CMe_2.CH_2.C_6H_{10}.OH$	146–7	B	18			76.1	76.2	11.9	11.9			
$HO.C_6H_{10}.CMe_2.CH_2.CH_2.C_6H_{10}.OH$	Liq.	A	47							165–9°/ 0.3 mm	1.4903 (25°)	96%

*The yield of the coupling reaction is given in methods A, B, and C. The hydrogenation steps of methods C and D and the oxidation step in method D were almost quantitative. The yield recorded for method D is for the ethynylation step.

†1,1′-Ethynylenedicycloheptanol (1,4-dihydroxy-1,1,4,4-bishexamethylene-2-butyne) had m.p. 81–2° (literature, 10, 78–80°).

‡1,1′-Diacetylenedicyclopentanol (1,6-dihydroxy-1,1,6,6-bistetramethylene-2,4-hexadiyne) had m.p. 136–7° (literature, 14, 133.2–134.2°).

§M.p. (thermometer immersed) 8°.

‖M.p. (thermometer immersed) 5°.

¶Hydrogenation equivalent, expressed as a percentage of the theoretical uptake for the diolefin.

Method B (18) was the conversion of a cyclic ketone to the symmetrical diol by reaction with the double Grignard reagent derived from an α,ω-dihalide having from 4 to 10 carbon atoms in the chain. In agreement with Lukes and Blaha (5), good results were obtained in this range, but it is generally recognized that aliphatic dihalides having three carbon atoms or fewer between the halogen atoms are useless in Grignard syntheses (6). We have, however, converted 1,3-dibromo-2,2-dimethylpropane (7) to a Grignard reagent and obtained a condensation product from it with cyclohexanone. The success of this reaction is the more remarkable in the light of the reaction of the dihalide with zinc (8) which gives dimethylcyclopropane in 96% yield. Whatever the reasons for the general failure of trimethylene halides in Grignard reactions, cyclopropane ring closure is evidently not one of them. In this instance it is suggested that the 2,2-dimethyl group functions either to limit polymeric coupling of the dihalide by steric hindrance or to preclude olefin formation as there are no hydrogen atoms α to the halogen.

Method C was the interaction of ethynylenemagnesium bromide ($BrMgC{\equiv}CMgBr$) with 2 moles of the appropriate cyclic ketone. Using cyclopentanone, cyclohexanone, and cycloheptanone, this reaction has been investigated previously (9, 10, 11, 12); we have used cyclooctanone also. The 1,1′-ethynylenedicycloalkanol thus obtained is hydrogenated in good yield to give the 1,1′-ethylenedicycloalkanol. It is possible to arrest the hydrogenation of the acetylenic diol in each case to give the olefinic derivative, but complete hydrogenation may be ensured by using either platinum or palladium catalysts in preference to nickel or by performing the hydrogenation over nickel at high pressures. The use of high temperatures in the hydrogenation must be avoided because the tertiary diols are susceptible to dehydration.

Method D, which also involved acetylenic intermediates, was confined to those diols having four methylene groups between the rings. By oxidation of 1-ethynylcycloalkanols, coupling of two molecules to give a diacetylene derivative results (13, 14, 15). This may be hydrogenated to a 1,1′-tetramethylenecycloalkanol. Both steps are practically quantitative.

All of the polymethylene diols were obtained as crystalline solids. One of the diols with a branched connecting chain, the symmetrical one, was also solid, but its unsymmetrical isomer was liquid. Many tenaciously retained sticky impurities, which were best removed by chromatography, and from the nature of the preparative methods adopted, one may infer that these impurities were polymeric. Attempts to prepare crystalline derivatives were unsuccessful, for the customary reagents for alcohol characterization are acidic and need heating. Under such conditions, dehydration of the diol results.

A detailed study of dehydration of ethylene and trimethylene diols of this series is still in progress. Members with four or more carbon atoms between the rings were found to give the expected olefinic dehydration products, and it may be assumed that they are of the alkylcycloalkene type rather than the less stable alkylidenecycloalkane type. It has been reported (4) that the product of dehydration of 1,1′-ethylenedicyclopentanol is also of this nature, although in this compound the presence of an α,δ-diol system might be expected to give a product with a butadienoid system, namely dicyclopentylidene-ethane. Previous studies also indicate that the dehydration of such an α,δ-diol might give a cyclic ether rather than a diolefin; for example, Henry (16) obtained 2,2,5,5-tetramethyltetrahydrofuran from 2,5-dimethylhexane-2,5-diol and Reppe (17) reported the conversion of 1,1′-ethylenedicyclohexanol to 7-oxaspiro-(5,1,5,2)-pentadecane. The quantitative microhydrogenation data on our dehydration products from both 1,1′-

ethylenedicyclohexanol and 1,1′-ethylenedicyclopentanol indicate that a mixture of diolefin and epoxide was obtained.

EXPERIMENTAL

All melting points, except where otherwise noted, were determined by the capillary-tube method on a thermometer calibrated against an Anschütz set with a National Bureau of Standards certificate. Melting points and other temperatures are recorded as degrees centigrade.

1,1′-Octamethylenedicyclohexanol (Method A, cf. ref. 4) and its Dehydration

Pentamethylenedimagnesium bromide and dimethyl sebacate were condensed by the known procedure (4) but benzene was added to the reaction mixture and it was worked up with ammonium chloride to minimize dehydration loss. The crude product was sticky and melted between 60 and 80°. It was adsorbed from benzene on alumina and eluted successively with benzene and diethyl ether. Crystals were deposited by evaporation of each eluate and these were united and crystallized once from heptane, m.p. 87.5–88°. Further elution of the column with ethyl acetate gave a viscous oil which was not further investigated.

The crystalline diol (7.0 g, 0.025 mole) was heated to 150° for 4 hours in the presence of iodine (10 mg). A further portion of iodine (5 mg) was then added and heating continued for 1 hour at 170°. The product, an oil, was taken up in ether and shaken with sodium thiosulphate solution. After drying, the solvent was removed and the residual oil distilled, yield 4.1 g, 67%, b.p. 157–161°/0.4 mm, n_D^{25}, 1.4911, m.p. (thermometer immersed) 5°. Other diols listed in Table I were dehydrated similarly.

1,1′-Ethylenedicyclooctanol (Method C)

Interaction of cyclooctanone and ethynylenemagnesium bromide (the Iotsitch reagent) (19) was achieved essentially by the method described by Pinkney and Marvel (11) for other ketones. Using ammonium chloride as described above for decomposition of the magnesium complex, 1,1′-ethynylenedicyclooctanol (1,4-dihydroxy-1,1,4,4-bisheptamethylene-2-butyne) was isolated in ether and finally obtained as a solid, m.p. 115°. Found: C, 77.8; H, 10.8, hydrogenation equivalent 132. Calc. for $C_{18}H_{30}O_2$: C, 77.8; H, 10.8%, hydrogenation equivalent 139.

By hydrogenation of the acetylenic diol in ethyl acetate as previously described, 1,1′-ethylenedicyclooctanol was obtained in nearly quantitative yield, m.p. 123–124°.

1,1′-Tetramethylenedicycloheptanol (Method D)

Ethynylation of cycloheptanone was performed in a manner analogous to the recorded ethynylation of cyclohexanone (20, 21). Oxidative coupling of this alcohol to yield the diacetylene derivative was accomplished by copper-catalyzed autoxidation in an experiment similar to that performed by Zal'kind and Aizikovich (13) on its lower homologue. 1-Ethynylcycloheptanol (70 g, 0.5 mole) in ethanol (75 ml) was added during 2 hours to a vigorously stirred solution of ammonium chloride (36 g), cuprous chloride (11.5 g), and hydrochloric acid (10 *N*, 2.5 ml) in water (100 ml) at 50° while a slow stream of oxygen bubbled through. The solution was stirred with oxygen passed in for 4 hours more, and then 250 ml of a saturated aqueous solution of ammonium chloride was added. Next day the solid product was collected by filtration and washed with ammonium chloride solution. It was then washed with hydrochloric acid (2 *N*) until the washings

were no longer colored. The crude product (70 g, an almost quantitative yield) melted at 164–166°. This was raised to 165–166° by one crystallization from ethanol.

This diacetylenic diol (2.5 g, 0.01 mole) was hydrogenated in ethyl acetate suspension (50 ml) over palladized calcium carbonate at ambient temperature and 32.5 p.s.i. Uptake was complete in 3 hours and the filtered solution deposited crystals of 1,1′-tetramethylenedicycloheptanol (2.5 g, quantitative yield, m.p. 80–84°). This product was stable to aqueous potassium permanganate and was purified by one crystallization from a mixture of petroleum ether and benzene, in which it is very soluble (m.p. 85–86°).

Diethyl 2,2-Dimethylglutarate

In our hands, the previously published procedures (e.g. ref. 22) for conversion of 2,2-dimethyl-4-cyanobutyraldehyde to 2,2-dimethylglutaric acid gave 55 to 60% yields of a sticky product melting at 55–60°. Such procedures employ nitric acid alone or mixed with concentrated sulphuric acid as the reagent for simultaneous hydrolysis and oxidation of the starting material and it is possible that side reactions of polymerization or further oxidation can take place. Much loss of material occurred during purification.

The method adopted resembled that employed by Bruson and Riener (23) for conversion of 2,2-diethyl-4-cyanobutyraldehyde to 2,2-diethylglutaric acid. 2,2-Dimethyl-4-cyanobutyraldehyde (24) was used as soon as possible after it was prepared, because it is unexpectedly susceptible to autoxidation and on one occasion an explosion occurred when a sample that had been kept for some months without precautions against peroxide formation was distilled. The cyanoaldehyde (60 g, 0.5 mole) was boiled with sodium hydroxide (25 g, 0.63 mole) in water (250 ml) until a homogeneous solution was obtained (½ hour). After cooling, a further 25-g portion of sodium hydroxide and a further 250 ml of water were added. Potassium permanganate (114 g, 0.7 mole) was ground to a slurry with water (1 liter) and added to the cooled, stirred solution at such a rate that the temperature was 35–40°. Following a further ½ hour of stirring, sulphur dioxide was passed until all the manganese dioxide was reduced. The 2,2-dimethylglutaric acid was isolated by ether extraction of the solution (66 g, 86%, m.p. 74–76°). By recrystallizing from a mixture of benzene and petroleum ether the melting point was raised to 83–84°, but the bulk of the product was esterified without purification, yielding diethyl 2,2-dimethylglutarate, b.p. 147/46 mm, $n_D^{22.5}$, 1.4262. (Found: saponification equivalent, 107. Calc. for $C_{11}H_{20}O_4$: 108).

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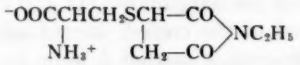
ADDUCTS FROM THE REACTION OF N-ETHYLMALEIMIDE WITH L-CYSTEINE AND WITH GLUTATHIONE

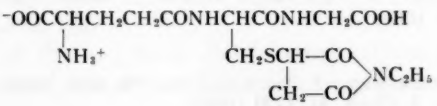
C. C. LEE AND E. R. SAMUELS

Since the first report of a quantitative reaction between thiols and N-ethylmaleimide (NEM) (1), a number of workers have described the use of NEM as a reagent for the assay of the sulphydryl group (2–6). However, it was only recently that the product from the reaction of L-cysteine and NEM was isolated and characterized as S-(N-ethylsuccinimido)-L-cysteine (I) (7). This note reports an independent isolation of I as well



(I)

as the preparation of S-(N-ethylsuccinimido)-glutathione (II), the adduct from the reaction of NEM with glutathione. Adduct II has not been recorded in any earlier publication.



(II)

The present work was undertaken as part of a project involving the determination of relative amounts of sulphhydryl and disulphide groups in certain proteins in which S^{35} has been incorporated. A suitable carrier for the reaction product between NEM and thiols was sought so that isotope dilution studies may be carried out. With this aim in mind, the behaviors of I and II on acid hydrolysis were studied. Somewhat surprisingly, it was found that after treatment for 18 hours in refluxing 6 *N* hydrochloric acid, I was recovered without change. The products from the hydrolysis of II were identified by paper chromatography as glycine, glutamic acid, and adduct I, exactly as expected for hydrolysis of the peptide bonds with I remaining unaffected. It was also noted that paper chromatographic separation of I from II, cysteine, and cystine can be easily accomplished. These observations indicate that it is likely feasible to use I as a

carrier in isotope dilution studies. If a protein containing S^{35} were allowed to react with NEM and then hydrolyzed in 6 *N* hydrochloric acid, any sulphydryl groups originally present in cysteine residues should give rise to S^{35}-labeled adduct I, which may be isolated with the aid of ordinary I as carrier.

EXPERIMENTAL

S-(N-Ethylsuccinimido)-L-cysteine (I)

Equal volumes of 0.1 *M* solutions of NEM and L-cysteine hydrochloride were mixed and allowed to stand at room temperature overnight. The resulting solution was concentrated under reduced pressure at 50–60° C. Ethanol was then added to precipitate the product. The latter was dissolved in distilled water, passed through a column packed with the anion exchanger, Dowex 1-x8, eluted with 0.5 *N* acetic acid, concentrated again under reduced pressure, and then crystallized with the addition of ethanol. The yield of purified S-(N-ethylsuccinimido)-L-cysteine, m.p. 194–195° C, decomp., was 85%. It gave a positive ninhydrin reaction and a negative nitroprusside test, which indicated the absence of the sulphydryl group.

Anal. Calc. for $C_9H_{14}O_4N_2S$: C, 43.89; H, 5.73; N, 11.38; S, 13.02. Found: C, 43.89; H, 5.78; N, 11.05; S, 12.90. This procedure for the preparation of I was essentially similar to that reported by Smyth and co-workers (7). However, it was noted that although, when the reactants were mixed, the pH was 1.8, it was not necessary to add sodium hydroxide to the reaction mixture as was done by Smyth *et al.* (7). It was found that inclusion of sodium hydroxide in the reaction mixture often gave a product containing detectable amounts of ash. The elimination of the base and final purification through the Dowex 1-x8 column proved to be quite satisfactory.

S-(N-Ethylsuccinimido)-glutathione (II)

Equal volumes of 0.1 *M* solutions of NEM and glutathione (reduced) were mixed and allowed to stand overnight. The resulting solution was worked up in the same way as in the isolation and purification of I. The product, S-(N-ethylsuccinimido)-glutathione, m.p. 204–206° C, decomp., was obtained in 90% yield. It also gave a positive reaction with ninhydrin and a negative test with nitroprusside.

Anal. Calc. for $C_{16}H_{24}O_8N_4S$: C, 44.44; H, 5.59; N, 12.96; S, 7.42. Found: C, 44.14; H, 5.59; N, 12.88; S, 7.13.

Acid Hydrolysis

One gram of I or II in 20 ml 6 *N* hydrochloric acid was refluxed for 18 hours. The resulting solution was concentrated to a small volume under reduced pressure, passed through a Dowex 1-x8 column, eluted with 0.5 *N* acetic acid, concentrated again and the product crystallized with the addition of acetone. After this treatment, unchanged I was almost quantitatively recovered. That I was the same before and after hydrolysis was established by mixed melting point; unchanged analyses for C, H, N, S; identical infrared absorption spectrum; and behavior in paper chromatography. Ascending paper chromatography on Whatman No. 1 paper with *t*-butanol – water – formic acid (69.5: 29.5:1) as solvent was run for 16 hours and developed with 0.1% ninhydrin in acetone. The same spot was obtained for I before and after hydrolysis. On the other hand, paper chromatographic analysis of the hydrolyzate of II gave three spots. By comparison with behaviors of known compounds, these spots were identified as glycine, glutamic acid, and adduct I.

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FINE STRUCTURE IN THE PROTON MAGNETIC RESONANCE SPECTRUM OF 6–6 NYLON

D. J. SHAW[1] AND B. A. DUNELL

Correlation of the nuclear magnetic resonance (n.m.r.) spectra of polymers with their physical properties and mechanical behavior has received considerable attention recently and has been reviewed by Slichter (1, 2) and by Sauer and Woodward (3). The existence of a narrow component of the n.m.r. spectrum superimposed on the broad line above certain temperatures has been associated with extensive molecular motion in the amorphous phase of such polymers as branched and straight-chain polyethylene (4–6) and isotactic polypropylene (7, 8). One might anticipate that a narrow component would also be observed in the n.m.r. spectrum of nylon in the vicinity of room temperature, corresponding to the onset in the amorphous part of the nylon of molecular motion which gives rise to energy dissipation and dispersion of elastic modulus in mechanical experiments (3, 9, 10). Although Slichter (11) had already reported the variation with temperature of line width and second moment of nylon 6–6 and given no indication of the existence of fine structure in the spectrum at any temperature, it was decided to reinvestigate this substance. Woodward, Glick, Gupta, and Sauer (12) have published detailed results of the change with temperature of the n.m.r. line width and second moment of a number of polyamides, both thoroughly dried and containing various H_2O and D_2O contents. They do not, however, mention the existence of any fine structure in the spectra. Illers and Kosfeld (13) have recently obtained evidence of a narrow component in the n.m.r. spectrum of a completely dry sample of nylon 6–12.

EXPERIMENTS AND RESULTS

The nylon used in this work was a multifilament commercial yarn containing no delustrant. It was extracted with boiling ether for 8 hours and dried in a vacuum desiccator over phosphorus pentoxide for 24 hours before any experiments were done on it. Initially, this nylon was sealed, without further treatment, into a sample tube under dry nitrogen and its n.m.r. spectrum taken with a broad-line spectrometer of the marginal oscillator type (14), operating at 28 Mc/sec, and a 6-in. Varian electromagnet.

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With this spectrometer the line widths obtained in the range from liquid nitrogen temperature to 400° K were substantially the same as those reported by Slichter (11), and no evidence of fine structure was obtained. The comparison of line widths is shown in Fig. 1. The widths that we obtained at the three highest temperatures are too great because of instrumental difficulties.

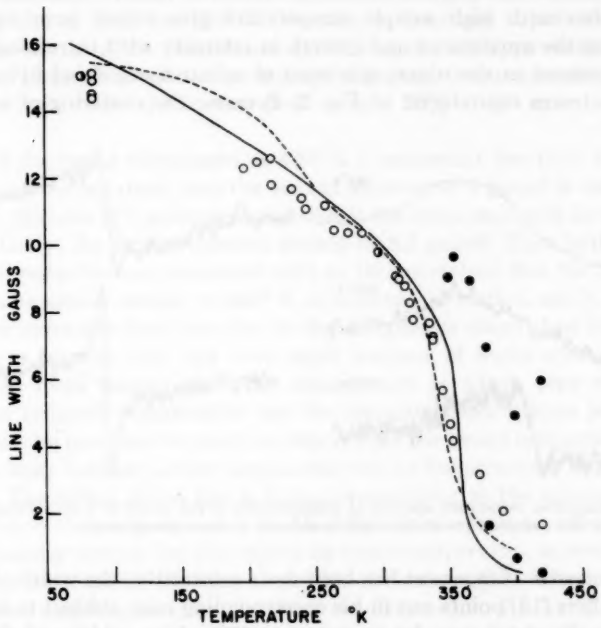

FIG. 1. Nuclear magnetic resonance line width of nylon 6–6 as a function of temperature. ○ This work, using sample dried at room temperature. ● This work, using exhaustively dried sample. ◑ Coincidence of open and filled points. ——— Results of Slichter (11). – – – Results of Glick *et al.* (12).

Since the α' dispersion region (found near 350° K for dry nylon 6–6) moves to lower temperature as nylon sorbs water, and is centered near room temperature when the relative humidity approaches 100% (10); evidence of fine structure in the n.m.r. spectrum of nylon was sought at room temperature using a sample that was in equilibrium with a number of different partial pressures of water vapor. An intense narrow component was obtained under these conditions, but it disappeared when the nylon was soaked in 99% D_2O for several hours, dried in a vacuum system, and exposed to D_2O vapor in the sealed sample tube. The marginal oscillator spectrometer gave the line width of the deuterated and dried nylon sample as 10.2 gauss and of the deuterated sample exposed to saturated D_2O vapor as 9.7 gauss, both at 295° K. The corresponding second moments were 16.3 and 14.4 gauss^2.

A second group of experiments was done using a Varian V-4250 combination high-resolution and broad-line spectrometer operating at 40 Mc/sec. When we used this apparatus we found that samples which were dried under vacuum at room temperature showed a narrow component which may be attributed to residual sorbed moisture in the sample. Illers has found that complete drying of the nylon can be achieved only by

heating at 100° C or more under vacuum (15). When we heated under these conditions for 6 hours, this narrow component observed at room temperature on the Varian spectrometer disappeared. The exhaustively dried sample was then run in the Varian spectrometer at temperatures up to 420° K. The sample was heated in a small Dewar vessel which fitted inside the spectrometer insert-tube by a stream of heated air, and the temperature measured by a thermocouple (16). The rather small sample and large insert-tube together with high sample temperature give rather poor signal to noise ratio. Nevertheless the appearance and growth in intensity with increasing temperature of a narrow component in the n.m.r. spectrum of exhaustively dried nylon can be distinguished in the traces reproduced in Fig. 2. Because the resolving of each spectrum

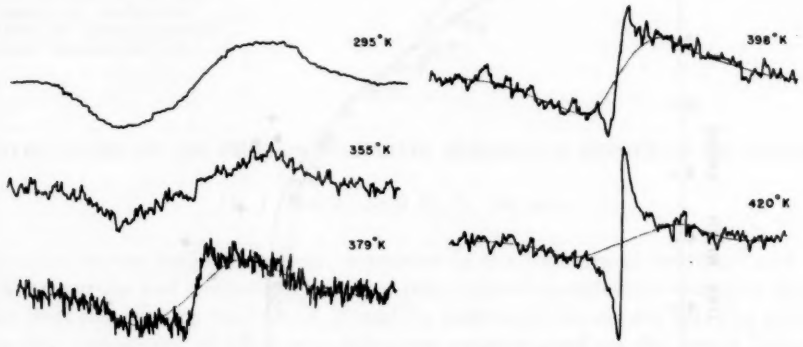

FIG. 2. Nuclear magnetic resonance spectra of exhaustively dried nylon 6–6 at various temperatures. The dotted lines show the assumed resolution into broad and narrow components.

into a broad and narrow component has been done arbitrarily, the width of the resolved broad line is, as Illers (13) points out in his corresponding case, subject to large error. It is estimated that the error may be as great as 40%. Line widths of the broad and narrow components are plotted in Fig. 1.

The deuterated sample was also re-examined on the Varian machine at room temperature. Dry, it gave a line width of 9.8 gauss and a second moment of 14.2 gauss^2; exposed to saturated vapor of D_2O, it gave 9.5 gauss and 13.0 gauss^2. These values are in satisfactory agreement with those obtained from the other spectrometer. A narrow component of very small intensity was observed with the deuterated system after the sample had been exposed to D_2O vapor for several days. This is attributed to traces of proton exchanging with sorbed heavy water.

The density of the nylon was measured in a density gradient tube (17) and used to obtain the percentage crystallinity from the correlation of Starkweather and Moynihan (18). The sample was found to be 35% crystalline.

DISCUSSION

In the exhaustively dried nylon the appearance of a narrow component near 380° K is attributed to the onset of very extensive molecular motion in part of the amorphous region of the substance. Such motion is considered to be the cause of the α' energy loss and dispersion mechanism (9, 10) which occurs at about 350° K at sonic frequencies in dry samples. Exhaustive drying would be expected to raise the temperature of the maximum in mechanical loss by several degrees (19), bringing it closer to 380° K. (An activation energy of 70 kcal/mole for the α' mechanism would correspond to a 20°

temperature difference between a correlation frequency of 10^5 for the n.m.r. experiment and a frequency of 650 c.p.s. (10) for the mechanical experiment.) As the temperature rises from 380° to 420° K the intensity of the narrow component increases, and its width decreases from 1.7 gauss (with modulation amplitude 1 gauss) at 379° K to approximately 0.3 gauss (modulation 0.12 gauss) at 420° K. The narrow-component line width indicated that although motion of the molecular chain segments becomes very extensive it is not liquid-like at least by 420° K. Further, it does not appear that even at this temperature the whole of the amorphous region is in the vigorous motion that produces the narrow line. Thus, although the percentage of amorphous component was found to be some 65%, the area under the narrow peak is only 20% of the area under both peaks at 420° K.

The width of the broad component at 420° K is somewhat less than half of the width at liquid nitrogen temperature, and the second moment of 6 gauss^2 is one quarter of the value at 77° K. Slichter (11) has calculated that if the chain segments in crystalline nylon 6–6 all rotate freely, the second moment should be 8.5 gauss^2. Thus both line-width and second-moment reduction are consistent with an interpretation that the part of the nylon which is not in extensive motion at 420° K is involved in motion which, on the average, is slightly more extensive than rotation of chain segments about their long axes.

When there is present even the very small amount of water remaining in a nylon sample dried at room temperature, the temperature at which very extensive motion begins must be reduced considerably and the amount of amorphous phase involved in the motion must be increased to such an extent that the broad component is masked by the now much more intense narrow component and no fine structure is distinguishable in the spectrum. The width of the line is that corresponding to the narrow component.

Our observation that exposure of a deuterated nylon sample to saturated D_2O vapor did not significantly reduce the line width at room temperature is entirely at variance with the results reported by Woodward and his co-workers (12), who found it decreased to about 1 gauss under approximately these conditions (i.e., 7.8 wt.% D_2O sorption at 295° K). We observed the same line width, 9.5±0.1 gauss, 1, 14, 15, and 28 days after exposure to D_2O vapor, using the Varian spectrometer; and, as mentioned earlier, this width agreed with that obtained from the other spectrometer using a different sample. The discrepancy would not seem, therefore, to be of diffusional, equilibrium, or instrumental origin. One must conclude that the difference of results arises from the fact that we have used a drawn-filament sample rather than "bulk polymer". In the drawn filament it is possible that the chain segments in the amorphous regions are restricted in mobility to such an extent, because of great crystallite to crystallite distance in the direction of stretching, that only the first traces of water can be effective in increasing molecular motion at given temperature.

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THE PROTON MAGNETIC RESONANCE SPECTRUM AND THE CHARGE DISTRIBUTION OF THE PYRIDINIUM ION*

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The protonation of pyridine by trifluoracetic acid has been examined by proton resonance techniques. In the presence of excess acid it has been found possible to observe the proton spectrum characteristic of the pyridinium ion. Figure 1 shows the spectrum of pyridine (in a 5 mole% cyclohexane solution) as well as those of trifluoracetic acid solutions containing 20, 10, and 5 mole% of pyridine respectively. A small amount ($\sim$ 3 mole%) of CH_2Cl_2 was added to each solution to serve as an internal reference signal. Modulation side bands of the reference at 140 and 240 cycles/sec are indicated on the individual traces. The assignment of the proton signals is based on the previously reported analysis of the pyridine spectrum (1). Figure 2 shows a more extended spectrum of the 5 mole% pyridine solution in trifluoracetic acid taken under conditions of lower resolution. It shows, in addition to the signal of the carboxyl group of the trifluoracetic acid and that of the α, β, and γ protons, a triplet signal characteristic of a proton bonded to a nitrogen atom. This confirms the presence of the pyridinium ion. The triplet splitting arises from spin coupling with the N^{14} nucleus having spin $S = 1$. The spin-coupling constant has the value 70 cycles/sec.

The position of the N—H proton signal, to the low-field side of the acid carboxyl group, is unusual for protons bonded to nitrogen. In neutral molecules this ordinarily appears at a much higher field. It is evident from Fig. 1 that there is also a low-field displacement, though smaller, for the signals of the protons of the pyridine ring when the ion is formed. Table I shows the actual chemical shifts (relative to CH_2Cl_2 internal reference) for the individual protons in pyridine and in the pyridinium ion. These shifts are necessarily approximate since an exact analysis of the pyridinium ion spectrum, which has broadened

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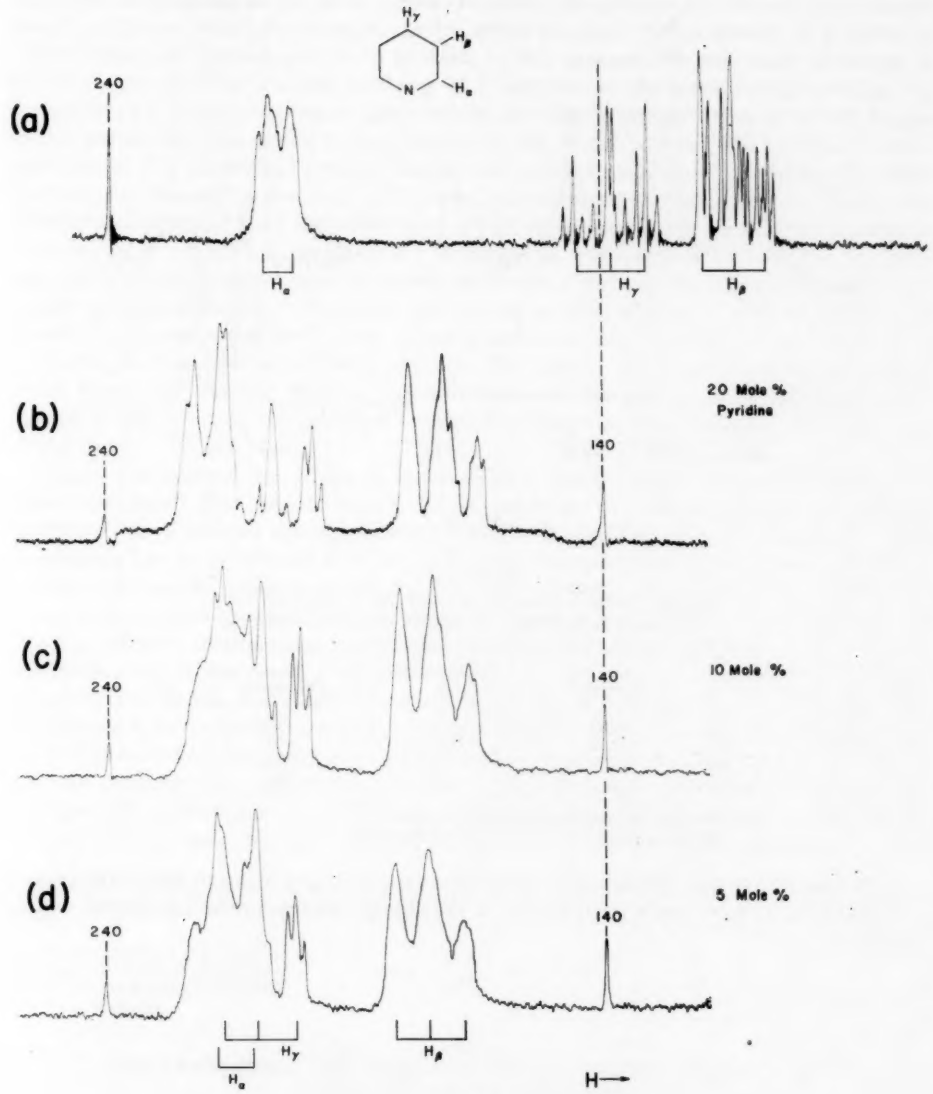


FIG. 1.

lines, is not possible. As shown by the differences listed in the last column, the largest low-field displacement occurs for the γ-proton, that for the β-protons is almost as large, while the corresponding displacement for α-protons is much smaller. These low-field displacements may be attributed to the decrease in electron charge density at the individual carbon nuclei as a result of adding the positively charged proton at the nitrogen atom. Assuming a simple proportionality between the proton resonance displacement

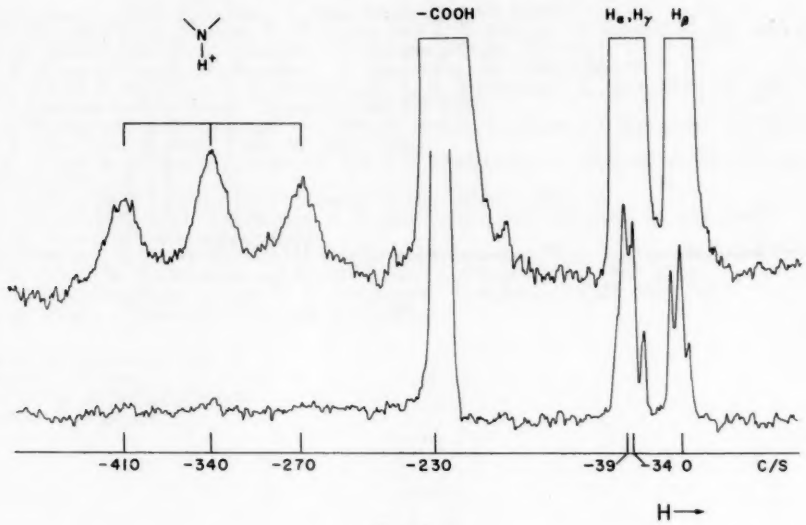


FIG. 2.

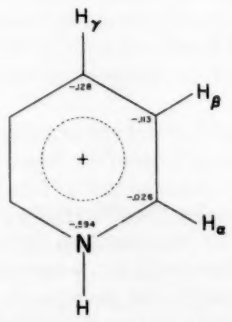
TABLE I

Proton chemical shifts of pyridine and pyridinium ion
(in cycles/sec at 60 Mc/sec relative to internal CH_2Cl_2)

	Pyridine*	Pyridinium† ion	Difference
H_α	−200	−215	−15
H_β	−112	−176	−64
H_γ	−137	−210	−73
N—H		−516	(−336)

*Measured in 5 mole% solution in cyclohexane.
†Measured in 5 mole% concentration in CF_3COOH.

and the electron charge deficiency on the carbon and nitrogen atom to which the proton is bonded* leads to a rough partitioning of the charge density in the pyridinium ion as follows.



A simple proportionality of this type is supported by a recent study of C^{13} and H^1 resonances in aromatic systems. (H. Spiesecke and W. G. Schneider. To be published.)

The local charges shown (in units of the electronic charge) are not the absolute charge densities, but represent the amount, $\Delta\rho$, by which the local charge density of pyridine is altered when the pyridinium ion is formed. In this process one unit positive charge is added to the pyridine nucleus resulting in a decrease in the local charge density, i.e. $\Delta\rho$ is negative. Employing the simple relation, $\Delta\delta = k\Delta\rho$, where $\Delta\delta$ is the observed change in the proton shift (in p.p.m.), the constant k was found to have the value 9.5 p.p.m. per electron. For the present purpose the low-field displacement of the N—H signal, resulting from the charge deficiency at the N atom, was taken to be 336 cycles/sec. This is the difference in chemical shift between the N—H group protons in the pyridinium ion and in pyrrole. The N—H shift in the latter is −96 cycles/sec relative to CH_2Cl_2 which, if pyrrole were an aromatic system, must be corrected for the "ring current effect" (2). This correction is approximately −84 cycles/sec, giving a total shift of −180 cycles/sec. On combining this with the N—H shift in the pyridinium ion, −516 cycles/sec, results in a net low-field displacement of 336 cycles/sec. The results, which are necessarily approximate, show that roughly 60% of the unit charge deficiency in the pyridinium ion is located at the N atom, the remainder being distributed on the carbon atoms in the ring as indicated.

Finally the relative line width of the resonance spectra under the experimental conditions employed are of some interest. In the spectrum of pyridine itself (Fig. 1(*a*)) the signals of the α-protons are significantly broader than those of β and γ protons. This broadening can be attributed to a shorter T_1 relaxation time of the α-protons caused by nuclear quadrupole relaxation effects of the N^{14} nucleus. Such effects are frequently encountered in nitrogen-containing compounds when a proton is directly bonded to the N atom, whereas in this instance the relaxation is evidently transmitted to protons two bonds removed. In the presence of trifluoracetic acid (Fig. 1(*b*), (*c*), and (*d*)), the α-proton signals remain broad, and in addition the β- and γ-protons are broadened. This additional broadening is no doubt due to proton exchange between the acid and the pyridine nitrogen atom. The fact that the triplet structure of the N—H proton is observable in the 5 mole% solution indicates that under these conditions the exchange rate must be considerably less than 400 times per second. With lower acid concentrations the proton exchange rate is much greater than this and the triplet N—H resonance cannot be observed.

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THE REVERSIBLE ELECTRODE POTENTIAL OF THE SYSTEM U/UCl_3 IN MOLTEN CHLORIDE SOLVENTS

D. Inman* and J. O'M. Bockris

This electrode potential has been measured with respect to various silver – silver chloride reference electrodes by Gruen and Osteryoung (1), Hill, Osteryoung, and Perano (2), Flengas (3), and Inman, Hills, Young, and Bockris (4). The results, expressed as $E^\circ_{UCl_3} - E^\circ_{AgCl}$, along with the theoretical values calculated from the data given by

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Hamer *et al.* (5), for the pure compounds, are shown in Fig. 1. These results are somewhat discrepant. Recently the present authors have repeated their previous measurements with an improved technique in an attempt to clarify the position.

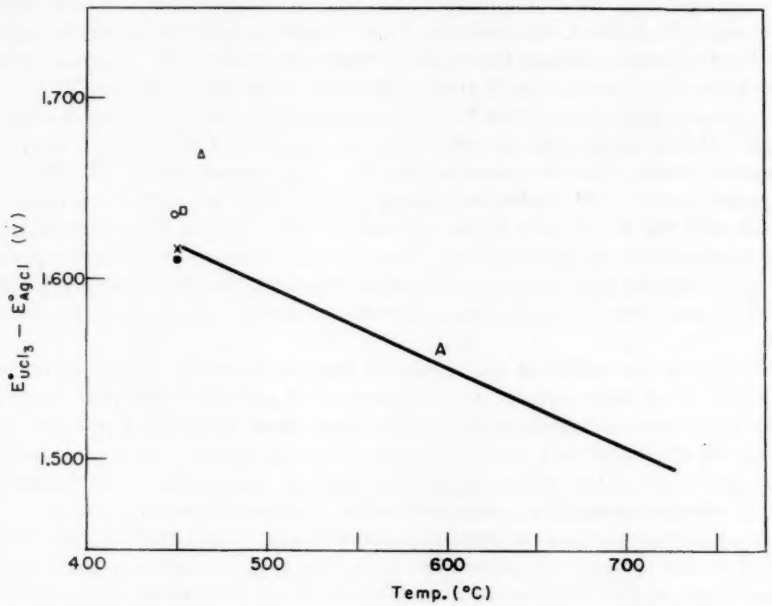

FIG. 1. $E^{\circ}_{(UCl_3)} - E^{\circ}_{(AgCl)}$ vs. temperature. (A) Electromotive force data of Hamer *et al.* (5). Experimental $E^{\circ}_{(UCl_3)} - E^{\circ}_{(AgCl)}$ values: △ Inman, Hills, Young, and Bockris (4). ● Hill, Perano, and Osteryoung (2). × Gruen and Osteryoung (1). ○ Flengas (3). □ Present work.

The experiments were performed at 453° C in a helium atmosphere. The LiCl–KCl eutectic solvent was purified by a modification (6) of the method described previously (4). The reference electrode was the same as that used before (7). The various concentrations of UCl_3 in LiCl–KCl were prepared by anodic dissolution of U wires into several similar glass diaphragm compartments to that used for the reference electrode. Both these compartments and the reference electrode compartment were immersed in a bath of purified LiCl–KCl eutectic.

The concentrations of UCl_3 in the separate compartments were determined by the dichromate method (8). A standard sample containing 1.025 mg of U was determined as 0.998 mg by this analytical method. The anodic dissolution process was ~100% efficient on the basis of $n = 3$, i.e., when 7.42 mg of uranium were expected in the compartment after the anodic dissolution, 7.60 mg were found.

The electromotive force results are shown in Table I.

A line of theoretical slope for $n = 3$ through these points gives $E^{\circ}_{(UCl_3)} - E^{\circ}_{(AgCl)} = 1.638$ v. This value is also shown in Fig. 1. (The previous value (4) at 463° C was 1.668 v.) The corresponding f_{UCl_3} values (standard state pure UCl_3 at the same temperature) are 3.8×10^{-1} and 0.8×10^{-1} respectively.

TABLE I

Electromotive force of the cell U^{-}/UCl_3, LiCl–KCl ¦ AgCl, LiCl–KCl/Ag^{+} at 453° C

e.m.f. (v)	N_{UCl_3}	$\log_{10}N_{UCl_3} - 3\ \log_{10}N_{AgCl}$	N_{AgCl}
1.472	$1.113.10^{-3}$	3.462	$7.27.10^{-3}$
1.450	$2.880.10^{-3}$	3.875	
1.431	$4.894.10^{-3}$	4.106	
1.416	$1.048.10^{-2}$	4.436	

The present result is seen to be in good agreement with the value obtained by Flengas (3) by extrapolation of his results obtained at a higher temperature. On the basis of the ionic sizes of Pb^{2+} and U^{3+} (9) the implied degree of complexing seems reasonable (10). The results of Gruen and Osteryoung (1) and Hill *et al.* (2), indicate positive deviations from ideality on the basis of the data of Hamer *et al.* (5).

ACKNOWLEDGMENTS

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